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CONTENTS

I. THE FREQUENCY OF PLURAL AND MULTIPLE BIRTHS IN INDIA

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INTRODUCTION

In the absence of compulsory registration of births and deaths in India or midwives' records, like Japan (Komai and Fukuoka, 1936) there is no other alternative but to fall upon the hospital data for the frequencies of plural and multiple births in this country. As it is not also counted during the decennial census in this country the hospital statistics alone can give us an idea of the frequency of this rare phenomena, knowing full well that the hospital cases are selected in nature and do not represent a random sample of the population.

Up till now only two papers (Das, 1934; Paul, 1943) have appeared which furnish to some extent an idea of the frequency of twin births in India. Sir Kedarnath Das (1934) dealt with only 12 Indian hospitals from the three presidency towns of Calcutta, Bombay and Madras. He did not mention anything of Gruelich's (1930) previous study on this problem in reviewing the position of the other foreign countries. Das drew an important ethnic conclusion from the data utilised by him. He showed that 'there is a distinctly greater tendency to twin formation in coloured races'. He was led to this conclusion on the basis of the fact that the Negroes of the U.S.A. have higher twin births than the local white population. While this fact of higher frequency of twin births among the Negroes of the U.S.A. than the Whites has been proved to be true (Hamlett, 1936; Cobb, 1942) it should not be lost sight of the fact that the Negroes of the U.S.A. are mostly mixed and the high frequency of twin births among the American Negroes can only be properly assessed after a comparison of the same with the frequency derived from the African Negroes. We badly need some data on the frequency of twin births from the African Negroes.

Das further derived a twin ratio of 1 : 43 from 3,669 births with 85 twins in Madras hospitals and found it much lower than that of the Calcutta Bengalis with a ratio of 1 : 59, derived from 35,460 births with 615 twins. He attributed this to a 'distinct preponderance of twins in dark races' on the assumption that the Madras population is generally darker than the Bengalis. We will see later on how this contention does not hold in the light of larger data. On the contrary, we have found that the twin frequency in Bengal (1 : 75·3) is higher than that of Madras (excluding Mysore) (1 : 84·7). Unless some data is forthcoming from the African Negroes or from the aboriginal population of India it is premature to say that the darker races have a higher twin frequency [1] than the less coloured ones.

[1] Mention may here be made of our efforts to determine the frequency of twin births among the Maler of the Rajmahal Hills (Sarkar, S. S., The Reproductive Life of the Maler Women, *Man in India*, 24, 1944).

Paul (1943) recorded for his hospital[1] (National Medical Institute) twin births during the years 1938–42 and found a ratio of 1 : 68·6.

It is impossible to say anything regarding the racial distribution of twins in India without a careful analysis of the hospital records. The latter are rarely perfect in nature and in spite of Das's emphasis on the ethnic bearing of twin births there has been no attempt at accurate recording of details of the mothers delivering twins in some of the Calcutta hospitals. It is, however, plausible that except in the cosmopolitan cities most of the small town hospitals represent the native population and the twin ratio can be counted as a true ratio of the population.

The Data

With a view to ascertain the frequency of twin and multiple births in this country 69 questionnaires were sent to the various Indian hospitals and maternity homes. Of these 24 were sent to Bengal institutions only. The data are shown in Tables I and II. Dr. Nichols of the Bacteriological Institute, Colombo, very kindly provided the Ceylon data.

It will be seen from Table I that the hospital figures supplied at our request have been compared with the very useful maternity statistics published annually in the *Journal of the Association of the Medical Women in India.* It was soon found out that the hospitals are not uniform in their definition of the term 'total births'. Some include abortions in it and some do not. It will be clearly seen in the case of the Jubilee Memorial Hospital, Khamgaon, and Holdsworth Memorial Hospital, Mysore, who do not include abortions within total births and there are, probably, a large majority in this category. Some hospitals, for instance, the Duchess of Teck Hospital, Patna, (as seen in the figures for 1938–42), the D.J.Z. Hospital, Srinagar (as seen in the figures for 1936–40), the Daga Memorial Hospital, Nagpur, Cama and Albless, Bombay, the S.M.V. Hospital, Surat (as seen in the figures for 1936-37), include abortions within total births. The A.M.W.I. returns give separate figures for abortions and deliveries and as such it was possible to check a majority of the hospital data supplied to us. This enabled us to arrive at the number of total pregnancies from which the twin ratio has been derived. Unfortunately, this could not be done uniformly in the case of the Bengal hospitals. The Lady Dufferin Hospital, Calcutta, alone participates in the A.M.W.I. returns.

The correct twin ratio is derived from the total number of pregnancies. Gruelich followed the same in working out the ratios for the different countries, which is surely the basic study on this particular aspect of the problem.

It will also be seen that there are discrepancies between the data supplied to us by the hospitals and those given in the A.M.W.I. returns. There are differences in all the data excepting those of the two hospitals, namely, the Jubilee Memorial Hospital, Khamgaon, and the Holdsworth Memorial Hospital, Mysore. We have, however, followed the method stated below to obtain the best result.

We have taken the number of twins and triplets given to us by the hospitals as final, because excepting 3 hospitals all of them have given details regarding the sex combinations of the twins and triplets. The A.M.W.I. returns mention as 'twins' during the years 1936–38 whereas in the returns of the successive years they are recorded under 'multiple pregnancy'. In correcting the figures for total births the hospital's manner of treating

[1] The actual ratio given in the paper is 67·5, which does not agree with the data supplied to the author by the hospital authorities (cf. Table II).

TABLE I

Statistics of Twin Births in Hospitals outside Bengal

Sr. No.	Hospitals	Town	Province	Period	(i) A.M.W.I. Returns: Total Births	Abortions	Total Pregnancies	Twins	(ii) Hospital data: Total Births	Twins	Corrected fig. total preg.	Ratio	Remarks
1	Ganesh Das	Shillong	Assam	1936-42	1,049	104	1,153	8	1,034	3	1,153	1 : 384·3	
2	Lady Dufferin	Quetta	Baluchistan	1942	113	37	150	2	108	2	150	1 : 75	
3	Duchess of Teck	Patna	Bihar	1938-42	2,626	270	2,896	30	2,838	38			
				1936-42					3,865	53	3,865	1 : 72·9	*
4	Cama and Albless	Bombay	Bombay	1936-42	17,759	2,338	20,097	244	20,650	250	20,650	1 : 82·6	*
5	Nowrosjee Wadia	,,	,,	,,	No data				32,749	445	32,749	1 : 73·6	
6	S.M.V.	Surat	,,	1936-37	650	81	731	11	736	10			
				1936-42					2,679	41	2,679	1 : 65·3	*
7	Mure Memorial	Nagpur	C.P.	,,	4,230	384	4,614	71	4,134	69	4,614	1 : 66·9	
8	Daga Memorial	,,	,,	,,	3,079	285	3,364	62	3,383	62	3,383	1 : 54·6	*
9	Women's	Chhindwara	,,	,,	659	83	742	14	not given	15	742	1 : 49·5	
10	Jubilee Memorial	Khamgaon	,,	1937-42	914	97	1,011	18	914	18	1,011	1 : 56·2	
11	Lady Hardinge	Akola	,,	1936-42	3,175	240	3,415	39	3,141	38	3,415	1 : 89·9	
12	S.B.M.W.	Shegaon	,,	1940	162	7	169	3	235	3			
				1936-42					1,299	15	1,299	1 : 86·6	
13	Lady Butler	Khandwa	,,	,,	No data				1,678	20	1,678	1 : 83·9	No data for 1938.
14	Victoria Zennana	Delhi	Delhi	,,	7,306	1,060	8,366	114	7,319	107	8,366	1 : 78·2	
15	D.J. Zennana	Srinagar	Kashmir	1936-40	2,145	145	2,290	46	2,288	46			
				1936-42					3,536	77	3,536	1 : 45·9	*
16	Rainy	Madras	Madras	,,	6,981	1,055	8,036	109	6,947	104	8,036	1 : 77·3	
17	Caste and Gosha	,,	,,	1942	No data				2,800	24	2,800	1 : 116·7	
18	Holdsworth Mem.	Bangalore	Mysore	1936-42	3,209	658	3,867	45	3,209	45	3,867	1 : 85·9	
19	Vani Vilas	Mysore	,,	1936-40 and 1942	29,042	3,112	32,154	325	28,964	314	38,441	1 : 98·6	Abortions for 1941—509.
				1936-42					34,820	390			
20	Victoria Zennana	Hyderabad	Deccan	1936-42	Figures could not be compared				20,769	265	20,769	1 : 78·4	
21	Lady Aitchison	Lahore	Punjab	1938-42	3,634	1,223	4,857	68	5,238	58	5,238	1 : 90·3	
22	Lady Willingdon	,,	,,	1937-42	No data				6,278	92	6,278	1 : 67·2	
23	Lady Reading	Simla	,,	1936-42	1,291	335	1,626	23	1,303	20	1,626	1 : 81·3	
24	W.C. Med. Coll.	Ludhiana	,,	1936-38 and 1941	5,229	363	5,592	33	5,201	14			
				1936-42					9,452	36	9,452	1 : 262·6	*
25	C.M.S.	Multan	,,	1936, 1938 and 1942	715	45	760	15	783	12			
				1936-41					1,585	17	1,585	1 : 93·2	*
26	Umaid	Jodhpur	Rajputana	1938-42	1,594	272	1,866	31	1,825	31			
				1936-42					2,283	39	2,283	1 : 58·5	
27	Lady Dufferin	Lucknow	U.P.	,,	3,298	734	4,032	55	3,304	51	4,032	1 : 79·1	
28	Lady Lyall	Agra	U.P.	1936-42	9,135	2,157	11,292	153	9,133	176	11,292	1 : 64·2	
29	Medical Coll.	,,	,,	1941-42	No data				717	4	717	1 : 179·3	
30	Lady Dufferin	Cawnpur	,,	1938-42	2,762	555	3,317	52	2,762	46	3,317	1 : 72·1	
					110,757	15,640	126,397	1,571	197,089	2,582	209,023	1 : 80·8	

Abortions included in Total Births.

abortions was first ascertained. When it was found that the abortions have not been included within total births, the figures for total births and abortions were taken from the A.M.W.I. returns and added together to find out the twin ratio. In the case of hospitals including abortions within total births the figures supplied by the former to us were taken into account. Only three hospitals, Lady Aitchison, Lahore, Cama and Albless, Bombay, and Mure Memorial, Nagpur, show the greatest discrepancies in their figures for total births.

The hospitals outside Bengal thus show 2,582 twins in a total of 209,023 pregnancies, which gives a ratio of 1 : 80·8 or 1·24%.

The Bengal data were obtained from 11 hospitals. The details are given in Table II below:—

TABLE II

Twin and Triplet Births in Bengal hospitals

Sr. No.	Hospitals	Town	Period	Total pregnancies	Twins	Ratio	Triplets	Ratio
1	Lady Dufferin ..	Calcutta	1935-42	10,796	126	1 : 85·7	1	1 : (103·8)[2]
2	Ramakrishna S.M. Pratisthan	,,	1934-42	10,354	102	1 : 101·5	1	1 : (102)[2]
		TOTAL ..	..	21,150	228	1 : 92·8	2	1 : (102·8)[2]
				Total births.				
3	Bankura Med. School ..	Bankura	1931-42	1,052	18	1 : 58	..	..
4	Astanga Ayurved ..	Calcutta	1936-42	571	7	1 : 82	..	..
5	Ranaghat C.M.S. ..	Ranaghat	1930-42	2,194	32	1 : 69	..	..
6	Baldeodas Mat. ..	Calcutta	1929-42	29,291	371	1 : 79	..	..
7	Chittaranjan Seva Sadan	,,	1926-42	26,416	413	1 : 64	6	1 : (66·4)[2]
8	National Med. Inst. ..	,,	1938-42	1,783	26	1 : 68·6	..	..
9	Campbell Med. School ..	,,	1936-42	5,573	52	1 : 107	..	..
10	Carmichael Med. College	,,	1932-42	12,260	208	1 : 59 1	1	1 : (111)[2]
11	Manicktala Mat. ..	,,	1940-42	4,030	30	1 : 134·3	..	..
		TOTAL ..	..	83,140	1,157	1 : 71·9	7	1 : (108·8)[2]
		All Bengal	..	104,290	1,385	1 : 75·3	9	1 : (107·7)[2]

The figures from the Lady Dufferin Hospital and the Ramakrishna Mission Sisumangal Pratisthan have been separately treated since in both the cases the abortion figures were available, which were added to the total births to find out the number of total pregnancies. As such they show a much lower frequency of twin births than that derived from the other hospitals. The frequencies derived from the grand total of the 11 hospitals show a ratio of 1 : 75·3, which is nearer to that derived from the total births (1 : 71·9) than that derived from the total pregnancies (1 : 92·8).

Discrepancies in the treatment of a twin birth as a single birth or a double birth were again found in Calcutta hospitals. The Carmichael Medical College and the Lady Dufferin Hospitals treat twin births as two births, whereas the Ramakrishna Mission Hospital treats it as a single birth. It is not known, however, how they have been treated in the case of other hospitals. in and outside Bengal. These discrepancies, together with

those mentioned before in the case of abortions should be minimised by a common agreement between the various hospitals, if we want to give our hospital data a true scientific value.

The majority of twins from Bengal hospitals are known to be either Hindus or Muhammadans, as will be seen from the following table:—

TABLE III

Distribution of Hindu and Muhammadan Twins in Bengal Hospitals

Hospitals	Hindu twins	Muhammadan twins	Others	Total
Ramakrishna Mission	101	0	1	102
Carmichael Medical College	192	16	..	208
Chittaranjan Seva Sadan	400	19	..	419
Manicktala Maternity	22	8	..	30
Baldeodas Maternity	351	20	..	371
Astanga Ayurved	6	1	..	7
Bankura Medical School	18	..	..	18
Lady Dufferin, Calcutta	60	34	32	126
TOTAL ..	1,150 (89·8%)	98 (7·7%)	33 (2·5%)	1,281

The Bengal hospitals, therefore, present cases of Bengali twins in the majority with a sprinkling of natives of other provinces. The twin ratio as will be seen from Table II is 1 : 75·3 or 1·33%, derived from 104,290 births with 1,385 twins. This figure is much higher than that of 1 : 59 found by Das given for 'Bengalese and other native Indians'. It appears that Das obtained a high ratio because of the smaller size of the sample used by him.

The Brahmans of Bengal show a high preponderance of twin births as is evidenced from the following birth statistics. The castes of the mothers were recognised from the definitely known surnames, such as, Chatterji, Banerji, Basu, Mitra, etc. The details are given below:—

	Carmichael College (1937–42)	Ramakrishna Mission Pratisthan	Lady Dufferin	Total
I. Definitely known Brahman surnames ..	27	38	12	77
II. Definitely known Vaidya surnames ..	4	5	5	14
III. Definitely known Kayastha surnames ..	5	9	5	19
IV. Other surnames, which may include the above three as well	88 (81+7*)	51 (42+9†)	104 (34*+70)	243
TOTAL ..	124	103	126	353

* Muhammadans. † Non-Bengalis.

Through the courtesy of Dr. Nichols the following data from Ceylon were obtained:—

TABLE IV

Twin and Triplet Births from Ceylon

Year	Total births	Twins	Triplets
1930	205,106	1,412	8
1931	199,170	1,284	13
1932	199,370	1,260	6
1933	209,032	1,316	13
1934	206,512	1,250	7
1935	192,755	1,008	6
1936	192,060	1,264	4
1937	216,072	1,262	11
	1,620,077	10,056	68

The figures for Ceylon are much higher than those we have for the whole of India. It was possible because Ceylon keeps a better statistics than that of India and since 1927 particular care is being taken for the registration of multiple births. The ratio derived from the above data comes up to 1 : 161·1 for twins and 1 : (154·4)[2] for triplets.

The twin ratio from Ceylon opens up some interesting points. The indigenous Ceylon population is not very much different from what we have in India. Linguistically the Sinhalese is akin to the Aryan group of languages, of which we have definite histories of migration, while the northern portion is mostly Tamilian in origin. For the sake of brevity the three ratios are arranged together below:—

TABLE V

Twin Births from Bengal, Ceylon and other Provinces compared

Locality	Total births or pregnancies	Twins	Ratio	Per cent
Bengal	104,290	1,385	1 : 75·3	1·33
Other Provinces ..	209,023	2,582	1 : 80·8	1·24
India	313,313	3,967	1 : 79·0	1·27
Ceylon	1,620,077	10,056	1 : 161·1	0·62

The Indian figures are insignificant in comparison to that of Ceylon. More data are wanted and unless the Indian figure is raised to at least a million births the above Indian ratio should be cautiously used. Das's theory of higher frequency of twin births in coloured races all the more falls to ground in the light of the largest Ceylon data.

Sex Combinations in Twins

The sex combinations of the twins and triplets for hospitals outside Bengal are shown in Table VI below:—

Table VI

Sex Combinations in Twins and Triplets

Sr. No.	Hospitals	Period	Total Pregnancies	Twins					Triplets					
				♂♂	♂♀	♀♀	*	Total	♂♂♂	♂♂♀	♂♀♀	♀♀♀	*	Total
1	Ganesh Das, Shillong ..	1936-42	1,153	2	..	1	..	3	..	..	..	..	..	..
2	Dufferin, Quetta ..	1942	150	..	1	1	..	2	..	..	..	1	..	1
3	Duchess of Teck, Patna ..	1936-42	3,865	17	20	16	..	53	1	..	..	1	.	2
4	Nowrosjee Wadia, Bombay	„	32,749	149	150	141	5	445	1	1	1	2	..	5
5	S.M.V., Surat ..	„	2,679	13	18	10	..	41	..	..	..	1	.	1
6	Mure Memorial, Nagpur ..	„	4,614	22	16	31	..	69	..	..	..	1	..	1
7	Daga „ „ ..	„	3,383	27	22	13	..	62	1	..	.	1	.	2
8	Jubilee „ , Khamgaon	1937-42	914	6	3	9	..	18	..	..	..	..	..	..
9	Women's, Chhindwara ..	1936-42	742	4	4	7	..	15	..	..	.	..	..	..
10	Lady Hardinge, Akola ..	„	3,415	10	15	13	..	38	..	..	..	..	..	..
11	Lady Butler, Khandwa ..	„	1,678	11	2	7	..	20	..	..	..	..	..	.
12	S.B.M.W., Shegaon .	„	1,299	2	11	2	..	15	..	1	..	..	..	1
13	Victoria Z., Delhi ..	„	8,366	42	30	33	2	107	..	..	1	2	..	3
14	D.J.Z., Srinagar ..	„	3,536	20	32	25	..	77	..	1	..	..	..	1
15	Rainy, Madras ..	„	8,036	40	31	30	3	104	..		1	..	..	1
16	Caste and Gosha, Madras	1942	2,800	7	14	3	..	24	..	..	..	..	..	..
17	Holdsworth Mem., Mysore	1936-42	3,867	19	12	13	1	45	..	..	..	1	1	2
18	Vani Vilas, Mysore ..	„	38,441	137	122	131	..	390	2	3	2	4		11
19	Victoria Z., Deccan ..	„	20,769	92	82	91	..	265	1	1	1	..	..	3
20	Lady Willingdon, Lahore	1937-38	1,896	9	12	13	..	34	..	..	..	..	.	.
21	Lady Reading, Simla ..	1936-42	1,626	5	8	5	2	20	..	..	..	..	..	..
22	W.C. Med. Coll., Ludhiana	„	9,452	13	10	13	..	36	..	..	..	..	..	..
23	C.M.S., Multan ..	„	1,585	6	4	7	..	17	..	..	..	..	.	..
24	Dufferin, Lucknow ..	„	4,032	19	16	6	10	51	..	..	..	..	..	..
25	Lady Lyall, Agra ..	„	11,292	72	46	58	..	176	..	..	..	..	..	..
26	Medical Coll., Agra ..	1941-42	717	4	..	..	..	4	..	..	..	..	..	..
27	Dufferin, Cawnpur ..	1938-42	3,317	15	10	21	..	46	..	..	..	..	..	..
	Total ..	..	176,373	763	691	700	23	2,177	6	7	6	14	1	34

* Denotes sex unknown.

The sex combinations of 2,154 pairs of twins from hospitals outside Bengal are—

		actual ratio
♂♂ ..	763	1·10
♂♀ ..	691	1
♀♀ ..	700	1·01
	2,154	

Applying Weinberg's differential rule to the above figures it is seen that 64·2 % of the twins are dizygotic and 35·8% of the twins are monozygotic. The ratio of ♂♂ : ♂♀ : ♀♀ is 1 : 1 : 1, similar to that found in other countries excepting Japan. The twins comprise of 2,217 boys and 2,091 girls which yield a sex ratio of 106·03.

The sex combinations of the twins from Bengal hospitals could be known in the case of three hospitals only. They are given below:—

TABLE VII

Sex Combinations of Twins from Bengal Hospitals

Hospitals	♂♂	♀♂	♀♀	Total
Carmichael Medical (1937–42) ..	40	42	39	121
Lady Dufferin, Calcutta (1935–42)	40	42	38	120
Ramakrishna M.S.P. (1934–42) ..	37	32	31	100
TOTAL ..	117	116	108	341
Actual ratio ..	1·01	1	0·94	..

The differential rule could not be used on such a small sample. It, however, does not show any deviation from the 1 : 1 : 1 ratio as found in the case of other provincial hospitals. The known sex combinations for the total number of Indian twins, therefore, come up to ♂♂—880, ♂♀—807, ♀♀—808, which gives a total number of 2,495 twins. The differential rule yields from the above figures 64·7% of dizygotic and 35·3% of monozygotic twins.

Out of the 116 dizygotic twins mentioned in Table VII the ages of mothers were known in the case of 84 twins. The frequency distribution of the twins according to the age of the mother is shown in fig. 1. It definitely shows the tendency of the ♂♀ twins being born at the higher ages of the mothers.

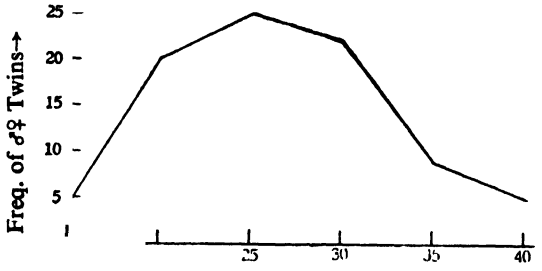

FIG. 1

Freq. distribution of ♂♀ twins according to mothers' ages

TRIPLETS

Thirty-seven triplets were reported from the hospitals outside Bengal. Their sex combinations are appended in Table VI. The sexes of three sets born in the Lady Aitchison Hospital, Lahore, were not known. Nine sets of triplets were reported from Bengal. The single triplet born in the Carmichael Medical College, Calcutta, was according to Mitter (1938) an uniovular set. It was born to a primiparous Bengali Hindu girl, aged 15.

The triplet was an all female set. The placenta showed no signs of fusion; it had a single chorion with three amniotic sacs separated from one another by complete septa. The babies were born alive but died later on. The single triplet from the Lady Dufferin Hospital was an all female set while that from the Ramakrishna Mission Hospital was an all male one. The latter was born to a primiparous, Bengali Hindu girl. Sixty-eight triplets were born in Ceylon (Table IV) during the years 1930–37. The triplet data are shown below:—

TABLE VIII

Triplet Births in India and Ceylon

Locality	Total births or pergnancies	Triplets	Triplet ratio	Twin ratio
Bengal	104,290	9	$1 : (107{\cdot}7)^2$	1 : 75·3
Other Provinces ..	209,023	37	$1 : (75{\cdot}2)^2$	1 : 80·8
India .. .	313,313	46	$1 : (82{\cdot}5)^2$	1 : 79·0
Ceylon	1,620,077	68	$1 : (154{\cdot}4)^2$	1 : 161·1

The sex combinations of the triplets reveal a high incidence of ♀♀♀ sets and also a significantly large number of girls over boys. Out of the 46 sets of triplets, 14 sets from the hospitals outside Bengal and 2 sets from Bengal are known to be ♀♀♀. The sex ratio derived from the sets of known sexes works up to 66 females to 39 males.*

QUADRUPLET

The single case of quadruplet birth was reported from the Lady Butler Hospital, Khandwa, C.P. This gives a ratio of 1 : 209,023 or $1 : (59{\cdot}33)^3$. The absence of any quadruplet birth in Ceylon, in spite of her larger statistics, is, however, worthy of mention. The above quadruplet was born to a Muhammadan (Bohra) woman of 23 years of age on 25th March, 1942. It was an all female set. It was a case of hydramnios and the first child was an anencephalic monster, which was born alive along with the other babies. All of them died subsequently.

ACKNOWLEDGMENT

My thanks are due to the Medical Officers and Superintendents of the various hospitals and maternity homes for their kind help in giving us the birth statistics of their respective institutions. Thanks are also due to the Secretary, Ramakrishna S.M. Pratisthan, Principal, Carmichael Medical College and the Medical Superintendent, Lady Dufferin Hospital, Calcutta, for their kindly allowing me to consult the birth registers of their institutions.

SUMMARY

1. In order to ascertain the frequency of twin and other multiple births in this country, statistics of twin, triplet and quadruplet births were collected from the various Indian hospitals.

* The triplet from the Holdsworth Mem. Hosp., Mysore, shown in Table VI as unknown consists of 2 females and a deformed male (?) child. Through the courtesy of Dr. Gillespie, the following details were available. It was a case of hydramnios in a 6th para Brahmin woman of 28 years of age. The deformed child was anencephalic, exomphalos, talipes, with adhesion from umbilicus to foot and round the ankle. There were two connected bags of membranes.

2. Statistics from 30 hospitals outside Bengal show 2,582 twins in a total number of 209,023 pregnancies giving a ratio of 1 : 80·8. Eleven Bengal hospitals yield a ratio of 1 : 75·3 derived from 1,385 twins in 104,290 total births. Ceylon presents a ratio of 1 : 161·1 derived from 10,056 twins in 1,620,077 total births.

3. The ratio for India has been found to be 1 : 79·0 or 1·27%, which should be cautiously used in the face of the Ceylon ratio, which is derived from a larger sample.

4. Sir Kedarnath Das's theory of 'a distinctly greater tendency to twin formation in coloured races' has not been found to be correct in the light of further data assembled in this study. More data are required from the aboriginal populations of India and the African Negroes before an ethnic relationship is interpreted.

5. Thirty-seven triplets from hospitals outside Bengal, nine from Bengal and sixty-eight from Ceylon were reported. The respective triplet ratios are 1 : $(75{\cdot}2)^2$, $(107{\cdot}7)^2$ and $(154{\cdot}4)^2$. The triplet ratio for India is $(82{\cdot}5)^2$.

6. One quadruplet birth was reported from Lady Butler Hospital, Khandwa, C.P.

7. Sex combinations of 2,154 twins from hospitals outside Bengal and 341 twins from Bengal are given. Applying Weinberg's differential rule it was found that 64·7% and 35·3% of the total number of twins are dizygotic and monozygotic respectively. The ratio of ♂♂ : ♂♀ : ♀♀ is 1 : 1 : 1 similar to that found in other countries, excepting Japan. The sex ratio derived from the total number of twins of known sexes from the whole of India works up to 105·9.

8. The triplets show an excess of ♀♀♀ sets and a large number of girls over boys (66 : 39).

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II. THE STRUCTURE OF THE PULVINUS OF *MIMOSA PUDICA* L. IN RELATION TO THE MECHANISM OF MOVEMENT

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INTRODUCTION

Mimosa pudica L. has been the subject of numerous investigations from time to time, but mainly from a physiological standpoint, as it exhibits an uncommon phenomenon, viz. the petiole drops and the leaflets fold together when the latter are touched. Hence the name, 'sensitive plant'. Most of these investigations centred round the conduction of the stimulus in the above and similar plants, the pioneer in the field being Sir J. C. Bose (1906, 1907, 1913, 1925*a* and *b*). Others who have made important contributions in this line are Ricca (1916), Herbert (1922), Kokestu (1923), Snow (1924 and 1925) and Dixon (1924). But the results of these investigations are rather contradictory. Dixon (1924) thinks that the xylem elements are responsible for the transmission of the stimuli. On the other hand, Herbert (1922) is of opinion that in the leaf of *M. pudica*, the phloem is the path of conduction. Snow (1925) agrees with Herbert in so far as the leaves are concerned, but opines that in the stem, the impulse is carried by the transpiration current. But according to Bose (1925*a*) 'the transpiration current has nothing to do with the conduction of the excitory impulse'. In a subsequent paper he (Bose, 1925*b*) tried to correlate the physiological data with the anatomical pattern. He showed that neither the transpiration current nor the movement of the sap were responsible for the conduction of the excitation in *Mimosa*. According to him 'the characteristic protoplasmic excitation at the cathode-make and at anode-break and the subsequent propagation of excitation to a distance, prove, on the other hand, that conduction in the petiole and in the stem of *Mimosa* is a phenomenon of transmitted protoplasmic excitation'. He assumes 'a nervous mechanism localised in the phloem of the vascular bundles' but does not clearly explain the path of the impulses from the exterior where the stimulus is usually given, as in his experiment of 'a drop of hydrochloric acid on the tip of the leaf of *Mimosa pudica*', to the region of the phloem. If his contention that 'the conduction is a phenomenon of propagation of protoplasmic excitation' is valid, then there must be some sort of protoplasmic continuity from cell to cell, especially in the cortical cells of the pulvinus, to discover which (if any), this work was undertaken.

PREVIOUS WORK

Gardiner (1883*a*) was the first to investigate the anatomy of the main pulvini of *Mimosa* by the 'staining method'. He treated the freshly cut pulvini with an aqueous solution of picric acid for 24 hours, rapidly washed in water and placed in alcohol till the yellow colour of the picric acid completely disappeared. Axial longitudinal sections were then taken and treated with sulphuric acid to swell up the walls and stained with iodine, methyl violet and glycerine. He was able to see in a 'well-prepared section where the action of the acid has been properly regulated, plain examples of continuity'. But this method results in plasmolysis of the cytoplasm to such an extent (refer to Gardiner's figure) that the protoplasm has an irregular stellate appearance. The observations on such preparations are far from satisfactory. In fact, according to Meeuse (1941) 'other cell wall structures have often been confused with plasmodesmata and, like artefacts, have

misled many investigators. Therefore many statements are doubtful and have been contested on good grounds, rendering many papers published before 1937 worthless'. Therefore the present investigation was mainly directed towards finding another technique, against which this criticism could not be levelled.

Materials and Methods

The materials for the present study were grown in pots at the Bose Institute and were well watered the day prior to fixation. The next morning (at about 9-30 a.m.), the main pulvini were cut off as quickly as possible with a sharp scalpel and kept in Navashin's fixative (Muntzing's modification) for 24 hours. The materials as a rule sank to the bottom in the fixing bottle. An evacuating pump was not used to prevent the displacement of the nucleus which may be caused by the rapid removal of the air from the tissues. The pulvini which did not sink in the fixative were discarded. The fixed materials were washed in tepid water for about 3 hours and graded up the lower grades of alcohol and left in 70% for 4 days to harden the tissues so as to prevent any distortion in cutting or subsequent treatments. They were then graded up the alcohol and chloroform series and embedded in paraffin. Sections were cut at 10μ thickness. Some of the slides were stained in light green, safranin and eosin while others were left unstained. The slides were dehydrated and cleared in the usual manner and mounted in canada balsam.

The present method is based upon the observation of the differential transmission of polarised light through the plant cell walls as compared to the cell body. The secondary wall of each plant cell is composed chiefly of long chains of cellulose molecules which are deposited in regular order on the cell walls and as such the walls are optically anisotropic. Under crossed nicols light passing through a transparent isotropic medium, like the cytoplasm of cells, will be completely quenched. Any anisotropic substance present in the field of view will restore a portion of the quenched illumination, and will appear as a bright patch against the dark background of the field of view. The cell walls appear as bright rings against a dark background. Any intercellular connections by means of protoplasmic threads will appear as dark breaks on such bright rings. The polariser and analyser used in the present observations were cut out of polaroid plates. These plates do not completely quench the transmitted light, which appear to be of deep red colour in the position of maximum quenching. The Leitz Ortholux Research Microscope used in the present observations was adapted for this purpose. The polariser was placed on the glass platform just above the reflecting mirror. The analyser on the other hand was cut, inserted in a brass ring and placed on the platform inside the eyepiece. My thanks are due to Dr. J. P. Sirkar for making the necessary adjustments to the analyzer to fit the eyepiece.

Observations

I do not propose to go into the details of the distribution and arrangement of the tissues in the main pulvini of *Mimosa* as they are sufficiently well known. The present observations are therefore confined to the cortical cells under polarised light.

Figure 1 is a transverse section of the cortical layer. Here the cellulose cell walls appear as white bands while the other portions of the cells are dark. A number of delicate lines are seen across the white bands. These naturally must represent gaps in the cell walls through which there may be continuity of protoplasm from cell to cell. In other words, they may be the paths of the plasmodesmata. These dark lines are not uniformly distributed around the cells. Some of the cell walls show a number of these lines, others fewer and still others none. Again these lines are not uniformly thick. Also, they may

be close together or far apart. Fig. 2 represents a portion of the cortical layer with intercellular spaces, which are shown as small dark triangles in the white strips. In this figure also, these dark lines are seen running across the white strips (cell walls), establishing a continuity between cell to cell. These again may vary in number, position and thickness as before. But they are not seen extending into the intercellular spaces. This is the usual rule. A careful examination was made to find out if any of these lines extended into the intercellular spaces also. Fig. 3 illustrates such a case. Here 5 dark lines (3 on one side and 2 on the other) are seen running into the triangular intercellular space. The middle lamella—non-cellulose—is seen as a dark line connecting the intercellular spaces. Fig. 4 is an extreme case where these dark lines are seen running into the intercellular spaces, in addition to the usual connection from cell to cell. Such cases are very rare.

Discussion

Plasmodesmata or the 'living threads of protoplasm connecting adjacent protoplasts through non-living substances (cell walls; mucilaginous intercellular substances as in *Volvox* colonies)' have been the subject of numerous investigations from time to time and a great deal of literature has accumulated on the subject. An excellent review of the literature has been published by Meeuse (1941), but for the sake of continuity, the important observations are briefly dealt with.

Tangl (1897) was the first to describe the connecting threads of protoplasm which pass through the walls of cells, although Hofmeister, Dippel and Goroschankin had observed these connections previously without recognising the importance of their observations (Meeuse, 1941). Subsequently a number of investigators such as Gardiner (1883*a* and *b*), Kienitz-Gerloff (1891, 1902), Meyer (1896), Strasburger (1901) and Livingston (1933) tried to show the presence of these connecting threads in plants.

Usually, plasmodesmata can be demonstrated only by special techniques. In the early days, these included too drastic swelling of the wall so that the results of many of the earlier investigators were unreliable. At the present time, the technique usually consists in soaking in iodine reagents with or without previous fixation, followed by swelling of the walls in zinc chloride solution or various concentration of sulphuric acid and then staining with some violet stain (Zimmermann and Schneider, 1922; Strasburger, 1923; Chamberlain, 1932; Muhldrof, 1937, etc.). In exceptional cases as in *Volvox aureus* (Meyer, 1896) or in the endosperms or cotyledons as in *Strychnos nux-vomica* (Tangl, 1879), *Diospyros* (Quisumbing, 1925), etc., these could be made out without any special treatment. In all other cases (except that of direct observations) the results cannot be relied upon as other cell structures and artefacts may at times be mistaken for plasmodesmata. Such cell structures are 'dermatose-like granules, caused by swelling agents often in linear arrangements and showing beaded or granular appearance', and very narrow pit cavities and variations of cellulose content in different parts of the wall. The threads of protoplasm, present after plasmolysis, between the wall and the contracted protoplast, especially if in adjacent cells they happen to lie in a straight line may also give the false appearance of plasmodesmata. In fact Gardiner's (1883) figures of plasmodesmata in *Mimosa* bear a strong resemblance to the latter and hence may not be real.

Then the question is, in those cases where direct observation is not possible, is there any better technique for demonstrating plasmodesmata? As shown above, all the staining techniques involve a certain amount of the swelling of the walls with the consequent distortion so that other structures also at times appear to be plasmodesmata. Therefore it is absolutely necessary that the technique employed should not involve the use of any

'swelling agents' or stains, if possible. From these points of view, the technique employed in the present observations, including the use of polarised light, appear to be well suited for the purpose. In the present observations, all attempts have been made to retain the cell structures in as near the living condition as possible, so that the results obtained may not be questionable. The extended use of this technique may lead to favourable results in other plants too, as according to Stanford (1934) although plasmodesmata have not been demonstrated in all plants, it is not improbable that they exist (sometimes on an ultramicroscopic scale) between all the adjacent living cells, thus knitting their protoplasts into an organised whole.

Acknowledgments

In conclusion, I wish to express my sincere thanks to Dr. D. M. Bose, Director of the Bose Institute, for providing me with every facility and for drawing my attention to the possibility of using polarised light for such investigations.

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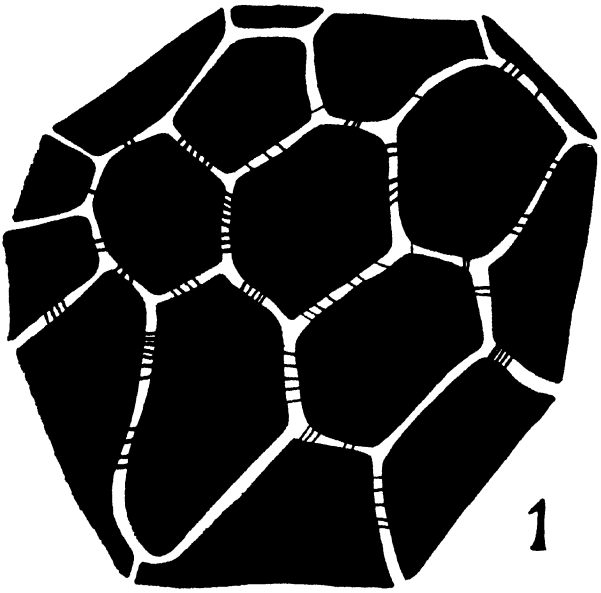
Explanation of Figures

All the drawings were made at table level with the aid of a Camera lucida and a Leitz Ortholux research microscope. A fluorite objective, A. 1.32 was used in conjunction with a periplanatic eyepiece ×6, giving an approximate magnification of 1,150 diameters. All the drawings were made from the transverse sections of the main pulvini of *M. pudica*, under polarised light.



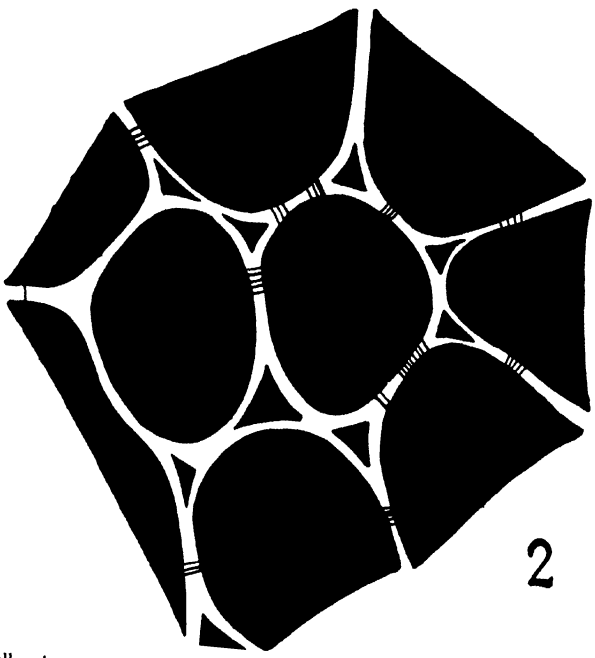
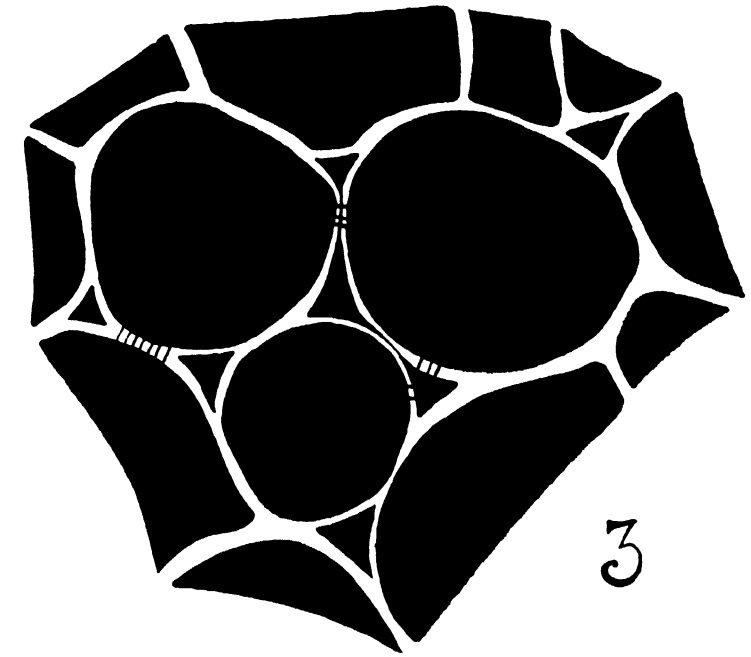


FIG. 1. Cortical cells, showing plasmodesmata, which are shown as thin dark lines, running across the walls shown as white bands.

FIG. 2. A portion of the cortex with intercellular spaces. Note that the plasmodesmata do not open out into the intercellular spaces.



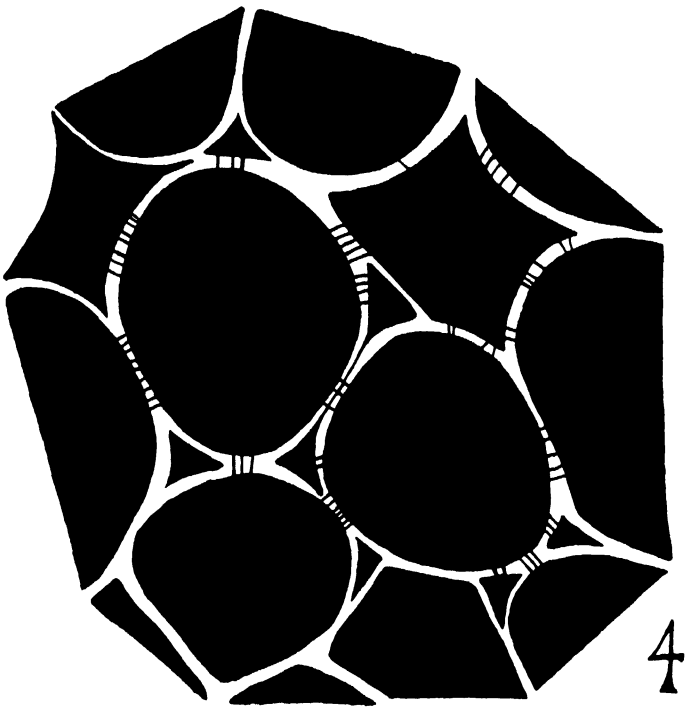


FIG. 3. A few cortical cells showing plasmodesmata In one case, they run into the intercellular spaces (shown as dark triangles); a rare case.

FIG. 4. A very rare instance in which these plasmodesmata are seen connecting the adjacent cells as well as the intercellular spaces.

III. DETECTION OF HORMONE CONCENTRATION IN PLANTS INDUCED BY THE ACTION OF HIGH CONCENTRATION AUXIN BY THE COLEOPTILE CURVATURE METHOD

By A. GUHA THAKURTA *and* B. K. DUTT

(*Received for publication, 3rd October, 1942*)

The effect of high concentration auxin on the growth of the coleoptile of Triticum has been dealt with in a previous paper.[1] It has been shown that in the coleoptile of Triticum when treated with high concentration auxin at the apical region, the growth is greatly increased, in comparison to the normal coleoptile and when treated at the basal region the growth becomes much less than the normal. To explain the cause of this diversely opposite reactions of auxin on the growth of the coleoptile it was suggested that auxin attracts the internal growth hormone from other regions to the region of its treatment and the concentration of the internal hormone induces growth changes in the treated region, depending upon the condition of the tissue of that region. In apical application concentration of internal hormone at the growing apical region enhances the growth, whereas, under basal treatment hormone is withdrawn from the growing apical region resulting in a cessation of growth; the basal portion having already become atrophied is incapable of further growth even when the internal hormone has been concentrated in that region. It was further shown in the same communication that the geotropism of the coleoptile can also be completely annulled under basal treatment of auxin when kept horizontal after a long time of treatment. The cause was attributed to the complete withdrawal of natural hormone from the growing region.

The increase of growth of the coleoptile by apical application of high concentration auxin was also observed by Went.[2] According to his conclusion the induced growth was due to the applied auxin. Hitchcock and Zimmerman [3] reported stunted growth of the plant by the application of auxin in the soil. The diversely opposite reactions of auxin in apical and basal applications was also shown by Le Fenu [4] in the pea shoots. She concluded that the upward inhibiting effect of auxin applied below is different from the accelerating effect of apical application of auxin. According to her the cause of inhibition of organ is not direct entry of auxin into it.

The effect of the diverse reactions of the apical and basal applications of auxin have been extensively studied in the shoots of Impatiens by the authors.[5] Similar to the observations in the Triticum coleoptile [1] and that of Le Fenu [4] in the pea shoots, the growth of the Impatiens shoot is accelerated by apical application and inhibited by basal application of high concentration auxin. Adventitious roots also appear at the treated regions after a few days. It was concluded that both the growth promoting and the root forming hormones, even if they are specifically different substances, are similarly attracted to the treated region from other parts of the organ. The inhibition of growth by basal application fully supports the former conclusion [1] that it is due to the withdrawal of growth hormone from the apical region. That the internal hormones are responsible for both growth promotion and root initiation have been proved from the ineffectiveness of the action of applied auxin in the absolutely defoliated stem; apical application of auxin in such stem induces neither growth increase nor root initiation. Later on it has

further been shown [6] that if the stem is split and one-half remains leafy and the other defoliated, roots can be grown on the defoliated side by treatment with auxin. This is a conclusive proof that the root forming hormone has been translocated from the leafy half. That the failure of root formation in the defoliated stem is not due to the shortage of food supply has also been treated in the same paper, in which it has been shown that the treated defoliated stem even when supplied with carbohydrates and vitamin B_1 failed to produce roots.

There is sufficient evidence in the above experiments to justify the conclusion that in auxin treated plants the growth changes or the root initiation is directly associated with the internal hormone and that the internal hormone from other regions is translocated to the auxin treated regions by the attraction of the auxin. But a direct and more convincing proof of this could be obtained by directly measuring the hormone concentrations in the plant. Even taking for granted that internal hormone becomes concentrated at the treated region, this cannot be proved by direct measurement of its concentration at the treated region, because under such condition there will be possibility of interference from the applied auxin which may itself produce an additive effect. The present investigation was therefore undertaken to detect if there is actual withdrawal of hormone from the untreated region of the treated plant, by directly measuring the concentration of that region. The experiment was conducted on Avena coleoptile. The basal portion of the coleoptile was treated with auxin and after twenty-four hours of treatment the hormone of the tip was measured by the coleoptile curvature method by unilateral application of the tip or its agar extracts. Normal concentration of hormone in the untreated coleoptiles was also measured in the same method and compared to those obtained in the tips of the treated coleoptile.

Materials and Methods

Coleoptiles for experiments were raised from a pure line seed of Avena sativa, I.P.1, obtained from the Imperial Agricultural Research Institute, Substation Pusa.

The seeds were freed from husks and germinated in petri dishes containing moist cotton. The seeds for germination were arranged unidirectionally to facilitate the straight growth of the coleoptile by placing the petri dish at a certain angle. In raising the test plants, i.e. the coleoptiles on which physiological tests were conducted, the seeds just after arranging in the soaked cotton were exposed to red light for twenty-four hours for inhibiting the growth of the mesocotyl, following the methods of Lange [7] and du Buy and Nuerenbergk.[8] It has been found that the increased growth of the mesocotyl under the ordinary method of germination, stands in the way of the straight growth of the coleoptile. After removing the germinating seeds from red light the petri dish with the seeds was kept at an angle of forty-five degree with the embryo side of the seed pointing downwards, in complete darkness. After another twenty-four hours, i.e. after forty-eight hours from the time of soaking, the seedlings, attaining a length of 1 cm., were mounted in glass holders similar to those devised by Went.[9] The mounted coleoptiles remained in the holders for another twenty-four hours when they attained a length of 3 to 3·5 cm. and became ready for experiment. Such coleoptiles were decapitated up to a length of 5 mm. and after pulling out the primary leaf a dried peduncle of grass was inserted into the coleoptile 2 mm. deep. The coleoptiles with the holders were arranged in a row on a wooden frame having a water trough at the bottom. After 100 minutes of decapitation, a time found to be most suitable by Schneider and Went [10] in physiological test for obtaining uniform curvature, the coleoptile tips from experimental plants for the detection of their hormone contents or agar blocks having their extracts, were placed unilaterally

on the cut surface of the test coleoptiles. The test coleoptiles in the row were divided equally in two groups; one of them receiving the tips of coleoptiles treated basally with high concentration auxin or their agar extract, and the other group, the tips of the control plants or their agar extract. The physiological reactions were recorded for ninety minutes in a specially made Photo-kymograph described later.

Experimental plant.—The coleoptiles of the experimental plants were raised from the same stock as that of the test plants. The dehusked seeds were arranged in the same way as previously described on moist cotton for germination. The soaked seeds for this purpose were not exposed to the red light because inhibition of the growth of the mesocotyl was not necessary. After twenty-four hours of soaking the petri dish with the germinating seeds was kept at an angle of 45° with the embryo side of the seed pointing downward. Next day, after forty-eight hours of soaking, the coleoptiles grew to a length of about 1·5 cm., when they were transferred to the holders mentioned before and some of them were treated basally just at the junction of the seed with 1% indole acetic acid in lanoline paste. After another twenty-four hours tips of 2 mm. length from both the treated and control plants were separately cut out either for direct application on the test plants or for extraction in agar blocks to be used afterwards.

Agar extraction.—In preparing agar blocks 3% agar solution was used. The blocks were of the size of 4 cu. mm. First of all an agar sheet of uniform thickness of 1 mm. was obtained. A detachable metallic frame with a rim of 1 mm. in height was arranged on a glass plate and 3% agar solution was slowly poured inside the rim. Another glass plate was pressed over it from above to squeeze out the superfluous agar. When the agar was cooled down and solidified the sheet of the requisite thickness of 1 mm. was obtained after carefully removing the glass plates and the frames. In cutting out the blocks from the sheet a specially made block cutter was used. A number of safety razor blades were fixed together with their edges parallel to one another having the intervening spaces in between two blades, of 2 mm. When the first incision was made by the cutter on the agar sheet a series of parallel strips were obtained; by a second incision at right angles to the former a number of blocks of the requisite size and shape were obtained. A number of blocks were separately kept in two covered petri dishes with the under side of the cover lined with moist blotting paper. One set was used for extraction of the tips from the basally treated coleoptiles and the other for the extraction from the tips of the control coleoptiles. The tips just after cutting were placed on the agar blocks and were allowed to remain there for two hours. After that the blocks were taken out and placed unilaterally on the test plants, as stated above, for recording their effects on them.

Photo-kymograph.—In determining the curvature of Avena coleoptile either the angle may be measured directly from the curving organ by means of a protractor or a shadow picture of the specimens may be taken on a photographic paper and the angles may be measured from the picture. But these two processes are very laborious, and in the latter case the specimens are subjected to light exposure which is not wanted. The photo-kymograph described by Schneider and Went [10] with some modifications was found to be suitable for the purpose of the experiment. A diagrammatic representation of the mechanism of the apparatus is given in figure 1. A metallic drum D, supported on a horizontal axis, is rotated by a clock-work arrangement C_1. Close to the drum a vertical sheet is placed; in this sheet there is 1 mm. slit S, of the length of the whole drum at the level of its axis. The coleoptiles held in glass holders are placed in front of this vertical sheet so that the straw heads inserted in the coleoptile tubes come in front of this slit, the coleoptiles remaining far below it; distance between the slit and the upper end of the

coleoptile was about 4 cm. When this whole arrangement is covered with a box (not shown in the diagram) another 1 mm. slit which is also at the same level as S, comes in

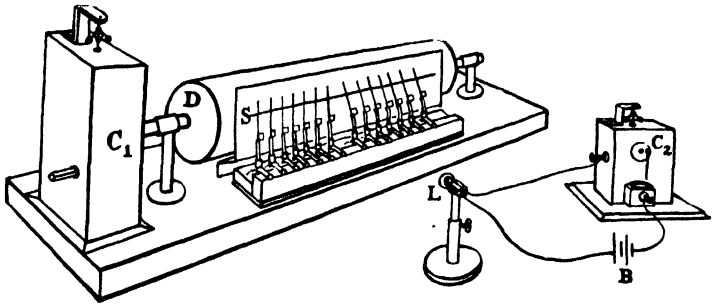


FIG. 1. Photo-kymograph for automatically recording curvature of Avena coleoptiles due to unilateral application of auxin. The drum, D, covered with photographic paper is rotated by the clock-work C_1. The series of decapitated Avena coleoptiles with grass peduncles inserted in them, are placed in front of the slit S. By covering the apparatus with a lid (not shown in the figure) another slit comes in front of the peduncles. An intermittent beam of light produced by the lamp L, battery B and clock-work C_2, passes through the two slits and strikes the drum.

front of the straws. The whole system now becomes light proof except at the level of the slits. An ordinary flash light lamp L, is placed in an adjustable stand in front of the box and in alignment with the two slits. With the help of a battery B and a clock-work arrangement C_2 a beam of light from L strikes the whole length of the drum covered with photographic paper, at three minute intervals. Thus the position of each plant is indicated as a white mark in the black line caused by the beam of light. The bending of the coleoptile is recorded by a shift of the white mark in the successive line records.

Experiment 1. *Determination of the hormone concentration at the tip of the treated plants by direct application of the detached tips on one side of the test plants.*

When the test plants as well as the experimental plants described above, were ready for experiment after seventy-two hours of the soaking of the seeds, identical specimens of the test plants were selected and arranged in the wooden frame with the glass holders and the water trough. According to the available specimens the number of plants employed in each experiment varied from 12 to 18 with half the number receiving the tips of normal

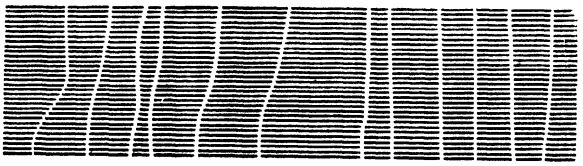


FIG. 2. A typical record from the photo-kymograph. Curvature of the coleoptiles is shown by the white marks in the black lines in the successive records produced at intervals of 3 minutes. Left six showing curvatures due to unilateral application of the tips of basally treated plants, and right six, those due to the tips of control plants.

plants and the other half receiving those of the basally treated plants. 5 mm. tips of the test plants were cut off and after pulling out the primary leaves dried grass peduncles 5 cm. in length were inserted 2 mm. in the coleoptile tubes. The plants with the projecting peduncle were placed in front of the slit *S* and a time interval of 100 minutes was allowed, after which 2 mm. tips of the experimental plants were cut off and placed unilaterally

on the cut surface of the test plants; half the number as mentioned before, received the tips of the control plants and the other half received those from the treated plants. Immediately after this operation the drum was started, the photo-kymograph was covered with the lid and the flash light was set to work at intervals of 3 minutes. A typical record is given in figure 2. The negative displacement of the projected coleoptile from the initial position after 90 minutes has been measured in mm. with a divider. Some of the test coleoptiles receiving the tips of the treated specimens were found to be slightly displaced in positive direction; those displacements were considered to be zero. Average displacements of the tips of the test plants due to unilateral application of the tips of treated and untreated plants are given in Table I; the results of 9 such experiments have been included in the Table. It will be found in the Table that the total mean displacement due to the tips of basally treated plants, is 1·05 mm. whereas, that due to the tips of untreated plants is 6·3 mm. This shows that the hormone content in the tips of untreated plants is six times greater than that in the tips of basally treated ones.

TABLE I

Determination of hormone concentration at the tip of coleoptile treated basally with high concentration auxin by unilateral application of tips of treated and untreated coleoptiles on Avena test plants

Record No.	Number of test plants used in each experiment with equal number in controls	Unilateral application of tips of plants treated basally with 1% indole acetic acid	Unilateral application of tips of normal plants
		Average negative displacement in mm. after 90 minutes	Average negative displacement in mm. after 90 minutes
I	8	2·9	13·2
II	6	0·3	7·6
III	7	0·85	1·9
IV	8	0·75	4·0
V	8	2·9	5·2
VI	9	0·0	6·9
VII	8	0·12	3·9
VIII	9	0·66	7·2
IX	8	1·0	7·2
Total mean of 71 plants		1·05	6·3

Experiment 2. In this experiment instead of directly applying the tips of the experimental plants to the test coleoptiles, agar blocks containing their extracts were applied on the test plants. 2 mm. tips of the treated and untreated coleoptiles were cut off and placed separately on two groups of agar blocks. These two groups of blocks were left for two hours in two covered petri dishes, the covers being lined with moist blotting paper in order to prevent drying of the tips and agar blocks. In the meantime, i.e. twenty minutes after the commencement of agar extraction, the test plants were prepared as described in experiment 1. 100 minutes after the preparation of the test plants and after two hours of agar extraction, the agar blocks were unilaterally applied on the test plants and record was taken as described before. Results of 10 experiments showing

the average displacements of the projected tips of test plants by unilateral application of agar blocks containing extracts of the tips of treated and untreated coleoptiles are given in Table II.

TABLE II

Determination of the hormone concentration at the tip of coleoptile treated basally with high concentration auxin by unilateral application of agar blocks containing hormone extracted from the tips of treated and untreated coleoptiles on Avena test plants

Record No.	Number of test plants used in each experiment with equal number in controls	Unilateral application of agar blocks containing hormone extracted from tips of plants treated basally with 1% indole acetic acid	Unilateral application of agar blocks containing hormone extracted from the tips of normal plants
		Average negative displacement in mm. after 90 minutes	Average negative displacement in mm. after 90 minutes
I	9	1	3·9
II	6	0·3	2·7
III	8	1·9	3·1
IV	6	0	1·0
V	8	1·3	2·1
VI	7	0·5	2·1
VII	6	0·3	1·2
VIII	9	0·1	3·8
IX	6	0	2·5
X	9	0·1	2·9
Total mean of 74 plants		0·55	2·53

DISCUSSION

From comparison of the figures of average displacements both in Tables I and II it will be quite conclusively proved that under basal treatment of auxin the hormone concentration at the tips is much lessened in comparison to that of the normal ones. In Table I the figure of the average displacement under normal tip is 6·3 and that under the tip of the basally treated plant is 1·05; the ratio of the capacity for displacement between the tips of the normal and basally treated plants is 6. In Table II the figures of displacements under agar extraction of the normal tip is 2·53 and of that of the basally treated plant is 0·55 mm., and the ratio of the capacity for displacement between the two is 4·5. The direct application of tip seems to induce comparatively much greater curvature than that induced by the agar extraction of the same. This is indicative of the fact that diffusion of hormone from the direct application of tip is much greater than that from the agar block having its extraction. Thimann and Bonner[11] found that only 13 to 21% of the growth substance present in the block is diffused in the plant. Went[12] found that as much as 86% of auxin from the block can enter into the plant when the block is small; with larger blocks, however, he found only 25 to 30% passing into the plant. But as it is not the usual practice in auxin determination to use small blocks for the difficulty of too much volume change by drying out, etc., the percentage of diffusion in normal

blocks can be taken as 15 to 30%, which are almost the same as the figures given by Thimann and Bonner. In the present experiment the ratio of the difference in efficiency between the tip and its agar extraction both in the normal as well as in the basally treated plant is approximately 2. This shows that the efficiency obtained by agar diffusion method is about 50% of that obtained by the direct application of the tip.

From careful examination of the data in Table I it will be evident that the intensity of response has varied within wide limit amongst different groups worked out on different days. This shows that there was considerable variation in the sensitivity amongst the different groups of plants. This variation has also been observed and broadly discussed by Schneider and Went.[10] According to them there can be seasonal, daily or even hourly variation in the sensitivity of Avena seedlings due to some as yet unknown external conditions. But whatever variation there might exist amongst the results of different groups the difference in reaction as obtained by the normal and the basally treated tip is quite marked in each of the individual groups.

The experiments have also thrown some light on the problem of the production of hormone in the coleoptile. It has been proved by different workers that the tip of the coleoptile produces auxin. Went [9] showed that only the extreme tip of Avena coleoptile, less than 0·7 mm. in length, produces auxin. Van der Weij [13] found that when cut off and placed on agar, the coleoptile tip continues to produce auxin for many hours; the production, however, finally drops to zero which according to him happens due to the exhaustion of the auxin precursor. In the present experiment the concentration of auxin in the tip of the basally treated coleoptile seems to have fallen very low in comparison to that of the normal tip within a period of only twenty-four hours of treatment. This shows that the applied auxin at the base of the coleoptile has not merely attracted the hormone from the tip but has done something more by which the tip has been exhausted of its auxin-producing capacity. This could possibly have happened by the exhaustion of the auxin precursor at the tip. The applied auxin at the base either induces rapid formation of auxin at the tip along with its rapid withdrawal and thereby inducing rapid exhaustion of the auxin precursor at the region or the auxin precursor is also withdrawn from the tip along with the produced auxin and concentrated at the treated region.

SUMMARY

The investigation was undertaken to detect if there was actual withdrawal of hormone from the tip of the Avena coleoptile basally treated with high concentration auxin by directly measuring the hormone concentration of the apical region. For detection of the withdrawl of hormone, concentration at the tip of the basally treated plant was compared to that of the control plant. The hormone concentration was determined by unilateral application of the tip or its extract in agar block on the test coleoptiles and by measuring the curvature with a specially made photo-kymograph. The curvature has been expressed as the actual displacement in mm. of the tip from its original vertical position.

By direct application of the tips of the control plants and those of the basally treated plants the average displacements of the test coleoptiles were found to be 6·3 mm. and 1·05 mm. respectively within 90 minutes.

By the application of the extracts in agar blocks from the tips of the control and the basally treated plants the average displacements were 2·53 mm. and 0·55 mm. respectively within 90 minutes.

It has finally been concluded from comparatively much smaller value of displacement produced by the tip of the basally treated plant that the hormone and possibly also its

precursor are withdrawn from the tip of the coleoptile by basal application of high concentration auxin.

Grateful thanks are due to Dr. D. M. Bose for his kind interest and helpful criticisms. Thanks are also due to Dr. J. P. Sircar for the design and construction of the apparatus.

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IV. VEGETATIVE PROPAGATION OF CINCHONA LEDGERIANA FROM GOOTES (MARCOTTE) AND CUTTINGS BY TREATMENT WITH AUXINS*

By A. GUHA THAKURTA *and* B. K. DUTT

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In Indian plantations propagation of Cinchona is made from seeds. The seedlings are raised in nurseries and when about two years of age they are transplanted in the suitable plantation plots. The plants are coppiced when 7 to 10 years old for extraction of quinine. The seeds for propagation are collected from plants in which quinine content is found to be high. But it has been found that, however high the quinine content might be in the mother plants its quality is not maintained in the next generation. This occurs as the inevitable result of cross fertilization. To obviate this difficulty grafting is practiced in Java. At present probably the whole of the Cinchona cultivation of Java is made from grafted plants. Varieties of Ledgeriana which is very rich in quinine content are inarched with Succirubra or Robusta stalks. In India also attempts were previously made to introduce this method for maintaining uniformity of quinine content in the cultivated stocks. But unfortunately this method could not be introduced in India because successful grafting was not possible. In explaining the failure Wilson et al [1] reported that it was mostly due to the ignorance of the correct technique and the want of perseverance in order to profit from previous failures. The problem, however, remains unsolved. No suitable method of vegetative propagation has yet been successfully found out for raising stocks of uniformly high quinine content.

The discovery of auxins and their judicious applications has considerably simplified the task of vegetative propagation of plants. It has been found by different workers that even the most difficult plants (i.e. plants which cannot be easily propagated vegetatively from simple cuttings or ring-bark cuttings) can be vegetatively propagated by application of auxins. Local application of auxin in stems initiates adventitious roots in that region. Vigorous root formation can be induced in cuttings or ring-bark cuttings by treatment with auxin, which under normal condition would form no root. Amongst the different methods of vegetative propagation inarching is comparatively more complicated than the methods of cuttings or ring-bark cuttings; the former requiring more skill and attention and more time in operation. It appears, therefore, desirable to replace the inarching by other methods which are simpler to use and promise an equal measure of success.

Previously in an attempt to find out a suitable method of vegetative propagation of mango other than by inarching, it was investigated whether propagation is possible from ring-bark cutting or goote and from cuttings by treatment with auxin.[2] There was vigorous root formation in ring-bark cuttings within 2 to 3 weeks by treatment with 1% indole acetic acid and 80% of the gootes were finally established in the soil. Cuttings also rooted vigorously by treatment with 3% indole acetic acid and some of them established in the soil.

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* Note published in 'Science and Culture'.

Soon after the publication of this report, Mr. S. C. Sen, Supdt., Cinchona Cultivation, Bengal, visited the Institute. He saw the mango plants raised from gootes and cuttings; they were vigorously growing in the Institute garden. He was very much interested in the work and highly appreciated its value in case of its application in Cinchona. Next he had a talk on the subject with Dr. D. M. Bose, the Director of the Institute and enquired whether the problem of vegetative propagation of Cinchona by method of auxin treatment, could be undertaken by the Institute. Dr. Bose gladly consented and asked the authors to take up the investigation. In order to conduct the work in Calcutta Mr. Sen sent to the Institute, in two instalments, some young Cinchona ledgeriana plants from the plantations at Mungpoo. The plants were sent in Summer and probably due to high temperature they all died after a month. Then it was decided that the authors should go to the plantations at Mungpoo and conduct the investigation there.

All the experiments reported in this paper were conducted at the Government Cinchona Plantation at Mungpoo in the District of Darjeeling, during the years 1941 and 1942.

Experiment 1. This experiment was undertaken in the month of June in the year 1941. Several concentrations (0·5%, 1% and 2%) of indole acetic acid and naphthalene acetic acid in lanoline paste were used in treating the gootes. It has been observed by Went [3] et al and later confirmed by Doak [4] that aneurin or vitamin B_1 is an accessory agent in the stimulation of root formation by hetero-auxin. According to this view some of the gootes were treated with vitamin B_1 in conjunction with auxins. The gootes were made in 8—12 year old plants, as well as in some 2 year old potted plants which were available at that time. The method of preparing the gootes and their treatment were similar to those used in the case of mango plants.[2] A ring of bark about one inch in length was peeled out of a portion in the hard wood branch so that the wood was completely exposed. The cuticle above the ring was slightly scratched with a knife and the wound was thoroughly washed with water. When it was dry the lanoline preparation of auxin was smeared round the portion above the ring to the extent of half an inch. After twenty-four hours the treated portion was covered with a lump of soil and dressed with coir.

In the mature plants 30 gootes were treated with each preparation of auxin, whereas, in case of two year old potted plants only 5 specimens were available for each preparation. Equal number of control experiments were also undertaken.

Results.—Three months after the treatment of the plants the results were first observed by Mr. S. C. Sen by opening the gootes. In the mature plants some of the gootes were found to produce a few roots in the treated portions. No roots were found to form in the control specimens. The gootes in the two year old potted plants were treated only with indole acetic acid preparations. Most of them produced roots ; in the case of 2% indole acetic acid the number of successful rootings was found to be considerable. In this experiment the control specimens were found to produce some roots. The details of the results are given in Table I.

The results of the first experiment were not very much encouraging. Still there were some points to be observed. The difference between the old and young plants as regards the possibilities of vegetative propagation was quite significant. In the young plants most of the gootes rooted both in the treated and untreated specimens though the best root formation was observed to have occurred in the plants treated with 2% indole acetic acid. But in comparison with the young plants only a few gootes in the old plants treated with high concentration auxin showed signs of root formation. In the previous experiment on the vegetative propagation of mango [2] too it was observed that the vegetative propagation is affected by the age of the plant. But on the whole the interest lies on the

propagation from fully grown plant. Because unless a plant is fully mature its true alkaloid content will not be known, and in raising an improved stock the quinine content of the mature mother plant must have to be first ascertained. The signs of root formation in the few auxin treated mature plants, however, indicated the possibility of root formation by treatment with proper auxin in proper concentration.

TABLE I

Root formation in the gootes of mature and young Cinchona plants by treatment with different auxin preparations in lanoline (observed for root formation three months after treatment)

Age of the plant	No. of specimens treated	Treatment	Result	
			Slight root formation in number of specimens	Profuse root formation in number of specimens
8–12 years	30	Indole acetic acid ·05%	..	..
	30	,, ,, ,, 1%	3	..
	30	,, ,, ,, 2%	4	..
	30	Indole acetic acid 1%+ Thiamin ·005%.	2	..
8–12 years	30	Naphthalene acetic acid ·05%.	2	..
	30	Do. 1%.	..	..
	30	Do. 2% ..	..	..
	30	Naphthalene acetic acid 1%+Thiamin ·005%.	..	..
8–12 years	30	Control ..	..	..
2 years	5	Indole acetic acid ·05%	3	..
	5	,, ,, ,, 1%	3	..
	5	,, ,, ,, 2%	..	4
	5	Indole acetic acid 1% + Thiamin ·005%.	3	..
2 years	5	Control	4	.

Our stock of auxin during the first experiment was very limited, so we could not study the effects of all the concentrations thought suitable, that year. Moreover, we could not procure indole butyric acid that year, which is one of the most potent auxin for root formation. So in 1942 when we had fresh supplies of auxins, we prepared an elaborate scheme to study the effects of different auxins in different concentrations both in water and in lanoline solutions. We also tried the effects of some vitamins and different amino-acids which have been observed by some workers [3, 4, 5, 6] to accelerate the effect of auxin in root formation in some plants. This experiment was limited to the cuttings only. The cuttings were dipped in ·02% water solution of indole acetic acid for twenty-four hours and were subsequently treated for the same period in 1 p.p.m. vitamin C or B_1 or 2% sucrose or in the mixture of the following amino acids: Glutamic acid 5 mg., Histidine 1·5 mg., Proline 0·5 mg., Valine 0·15 mg., Leucene 0·01 mg., and Isoleucene 0·0015 mg. In another experiment a treatment with 0·05% potassium permanganate for four hours was followed by a treatment with 0·02% indole acetic acid. The action of potassium permanganate has been found to accelerate the formation of root,[7] but its action is obscure; some suppose the permanganate acts in suppressing the growth of the micro organisms which inhibit root formation. Experiments were conducted on both the gootes and

cuttings on hard wood portions of mature plants. As there was no root formation in the untreated gootes in the previous year control experiments were not unnecessarily undertaken this year.

The results of the experiments undertaken in the year 1942 are given in the Tables II and III.

TABLE II

Root formation in the gootes of mature Cinchona ledgeriana plants by treatment with different concentrations of indole acetic acid and indole butyric acid in lanoline paste. (Observed for root formation, 4 months after the treatment)

No. of specimen treated	Treatment	Result		
		Profuse root formation	Slight root formation	Callus formation
25	Indole acetic acid 2%	2	4	8
25	,, ,, ,, 3%	1	1	11
25	,, ,, ,, 5%	2	3	13
25	Indole butyric acid 2%	20	2	3
25	,, ,, ,, 3%	15	4	3
25	,, ,, ,, 5%	7	3	8

TABLE III

Root formation in the hard wood cuttings of mature Cinchona ledgeriana plants by treatment with different auxin preparations. (Observed for root formation four months after treatment)

Treatment	No. of specimens	Result		
		Profuse root formation	Slight root formation	Callus formation
3% Indole acetic acid in lanoline for 24 hrs.	25	..	..	3
5% Do. do. ..	25	..	..	4
3% Indole butyric acid in lanoline for 24 hrs.	25	..	.	1
5% Do. do. ..	25	..	..	..
0·005% Indole acetic acid in water for 24 hrs.	43	..	..	4
0·02% Do. do. ..	43	..	..	1
0·005% Indole butyric acid in water for 24 hrs.	43	2	2	..
0·02% Do. do. ..	43	3	5	..
0·05% Do. do. ..	43	1	2	..
0·02% Indole acetic acid in water for 24 hrs. + 1 p.p.m. vitamin B_1 for 24 hrs.	25	..	..	..
0·02% Indole acetic acid in water for 24 hrs. + 1 p.p.m. vitamin C for 24 hrs.	25	..	..	..
0·02% Indole acetic acid in water for 24 hrs. + the amino acid mixture for 24 hrs.	25	..	..	..
0·05% Pot. permanganate for 4 hrs. +0·02% indole acetic in water for 24 hrs.	25	..	..	..
0·02% Indole acetic acid in water for 24 hrs. + 2% sucrose for 24 hrs.	25	..	..	..

It will be found from Table II that treatment with 2% indole butyric acid produced roots in 88% of the gootes and with 3% there was root formation in 76%. The root

FIG. 1. Root formation in the gootes of Cinchona ledgeriana by treatment with 2% indole butyric acid in lanoline paste.

formation was quite vigorous in most of them, which is shown in fig. 1. Vigorous root formation shown in the photograph sufficiently indicates that if such gootes are properly

FIG. 2. Root formation in hard wood cuttings of Cinchona ledgeriana by treatment with 0·02% indole butyric acid in water solution.

transplanted they are sure to establish in the soil. In the present case, however, we had to break open the bolls of earth for recording the results of the experiments and in doing so the root system was much injured which stood in the way of their subsequent survival. It will be found from Table II that indole acetic acid treatment from 2 to 5% induced slight root formation only in a few of the gootes.

In Table III treatment with 0·02% indole butyric acid in water solution induced root formation in 8 out of 43 specimens, i.e. in 18·6% of the treated cuttings. The other two concentrations of indole butyric acid, 0·005 and 0·05% in water solution, also induced root formation in a few of the treated cuttings. Figure 2 represents the specimens rooted by treatment with 0·02% indole butyric acid. The cuttings gave out new buds and were practically established in the soil. Had they not been taken out of the soil for studying their root formation they would sure grow into new plants. In taking out of the soil the tender roots were heavily mauled and having been exposed for a considerable time for observation they could not be re-established in the soil when planted. The treatments with high concentrations of both indole acetic and butyric acids in lanoline seemed to have little effect in producing roots in the cuttings. The treatments with sucrose, potassium permanganate and amino acid mixture produced no effect.

Discussion

We had to stop our work for unavoidable reasons, at an early stage of our investigation. But so far the experimental results are concerned it appears that the goote method can be successfully undertaken for the vegetative propagation of Cinchona. The advantage of vegetative propagation over that of seed propagation has been previously discussed. In the present plantations in India where seed propagation is in practice, there is great variation in the individual plants as regards to their quinine contents. In Ledgeriana alone which is the richest in quinine content of all the varieties of Cinchona, there may be variation from 4 to 19% in the individual plants of the same plot. If vegetative propagation is made from selected plants containing very high percentage of quinine and the plantation is made from those stocks only, production of quinine will be greatly increased per unit area in comparison to that obtained under the existing system. The technique of preparing the gootes is not so difficult and is much easier than that of inarching. If the whole plantation of Java can be made from inarched plants, there can be no conceivable difficulty of introducing goote system in Indian plantations so far the question of practicability is concerned. This can be done successfully by most ordinary labourers only trained for a couple of days. Cuttings represent a still more simple process of vegetative propagation and the results of our experiments on cuttings are not at all discouraging. There were rooting in about 20% of the cuttings by treatment with a concentration of indole butyric acid. The root formation was quite vigorous in some cuttings indicating that propagation can also be quite successfully made from cuttings if proper attempts are made. We could not devote much time on these experiments; we had only three short visits to the place for conduction of the experiments and observation of the results. If more time can be devoted to the investigation and proper post-treatment attention can be arranged, better results in the experiments of cuttings also are sure to yield.

For the last few years the import of quinine from outside having stopped, a great scarcity of this very important medicine has occurred in this malaria infested country. To cope with the situation an attempt is being made to increase the production of quinine by the Russian method of cultivation. Literatures of Russian method have not yet been

available to us, so we are not in a position to discuss in the present paper about its advantages and disadvantages over other methods. One great advantage of Russian method is, however, the time factor. In time of immediate necessity some quinine may be available by this method within 2 to 3 years of cultivation. In Russian method so far known, seeds are thickly broadcasted and when the seedlings are 2 to 3 years old quinine is extracted from the whole plant. But the quinine content in the seedling stage being exceedingly low, a very large area will be required to be brought under cultivation to get the requisite amount. The cost of production will also be comparatively large. In goote system also the total time of cultivation will be appreciably reduced. A goote at the time of plantation will be about the size of a 2 to 3 year old seedling. If the plants from seed are now coppiced for normal extraction of quinine at the eighth year of the cultivation, the goote plant which has gained an initial advance of two years, will require not more than 6 years to attain the same size and ready to be coppiced. Thus by adopting goote system the normal time of cultivation can be reduced by no less than two years and as they will be raised from uniformly high yielding plants, the yield per unit area can be increased by 100% or even more. Consequently a smaller area will be necessary to meet the requisite demand.

The initial expenditure of vegetative propagation from gootes or cuttings may be greater when compared to the present method of seed plantation, but it will be sufficiently balanced by the time factor. And when the yields are compared the higher initial expenditure of vegetative propagation will be sufficiently recompensed by its much higher total yield.

SUMMARY

Investigations were undertaken to find out a suitable method of vegetative propagation of Cinchona so that raising of uniformly high yielding stocks may be possible in Indian cultivation, where seed propagation is in practice.

Root formations were studied in gootes and cuttings by treatment with different auxin preparations and other accessory chemicals.

In gootes, treatment with 2% indole butyric acid induced vigorous root formation in 88% within four months of the treatment, the period of our observation.

In cuttings, treatment with 0·02% indole butyric acid in water solution induced root formation in 18·6% within four months of treatment.

The advantages of the vegetative propagation by gootes and cuttings over other methods, have been discussed and it has been concluded that the goote method can be profitably undertaken in Indian cultivation of Cinchona.

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V. EXPERIMENTAL STUDIES ON THE PARASITISM OF RICE BY *HELMINTHOSPORIUM ORYZAE* Breda de Haan AND ITS CONTROL IN FIELD AND STORAGE

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INTRODUCTION

Helminthosporium Oryzae Breda de Haan attacks rice in storage and paddy in the field, extensive wastage being recorded in Bengal and in certain parts of India in 1942-43. Nisikado and Miyake (1922) state that in seed beds the disease may attack about 90% of the seedlings and that sometimes all of the plants are infected. The disease has been recorded in Italy, Philippines, United States and in several other places. Sundararaman (1922) states that the *Helminthosporium* disease of rice produced a heavy damage in India in 1918-19. It is not known why there should be a heavy extent of wastage in a particular year but not in other years, although *H. Oryzae* may attack any part of rice plant at any time during the entire life of the plant in more or less varying degrees. The nature of the parasitism has been worked out by various workers (Ocfemia, 1923, 1924, Nisikado and Miyake, 1922, Sundararaman, 1922, Hemi and Matsuura, 1928, Nisikado, 1926), but so far the causes affecting the incidence of the disease remain as unexplained, as also the physiology of the parasitism of rice by this fungus.

Investigations were, therefore, undertaken to determine the mode of parasitism and the factors influencing the growth of the fungus in culture and on rice, especially with reference to its degree of specialization, and the control of wastage in field and in storage.

MATERIALS AND METHODS

H. Oryzae was isolated from the rice plants collected from various parts of Bengal as well as from stored grains. *Fusarium* sp. was also isolated in association with *H. Oryzae* from certain infected plants. Methods used for inoculating the plants are given below and the methods used for studying the growth rate of the fungus were the same as used by Baruah (1942).

HOST-PARASITE RELATIONSHIP

The conditions influencing the incidence of infection by *Helminthosporium* have been studied by various workers, but so far the degree of resistance or susceptibility of rice plants to infection has been determined by the extent of wastage (Ocfemia, 1924, Sundararaman, 1922, Nisikado and Miyake, 1922). The extent of wastage of rice by *H. Oryzae*, observed in the field and in storage conditions, does not, however, give a complete idea regarding the resistance or susceptibility of the rice plant or paddy seeds to disease in storage, because incidence of disease in an epidemic form or in less virulent form is not only influenced by the presence of number of spores in the field and in storage conditions, climatic conditions, water content of the soil, but by the more important factor, viz. the resistant and susceptible quality of the plant and paddy seeds. The following criteria were, therefore, adopted to determine the degree of resistance or

susceptibility of the plant to disease: (i) the percentage number of infections or infected plants and the extent of wastage in the field and in storage, and (ii) the time required for the development of the disease to a noticeable stage. Inoculations were, therefore, made on paddy seedlings, paddy seeds and the variation in degree of resistance of the plant to disease was studied.

Different varieties of paddy seeds are sown in pots on: (i) natural soil, (ii) water-logged soil, (iii) soil with infected seeds, (iv) water-logged soil with infected seeds; hundred seeds were sown in each pot. The total number of diseased plants are observed from time to time and the results after one month are recorded in Table I.

TABLE I

Total number of diseased plants after one month

Nature of soil	TOTAL NUMBER OF DISEASED PLANTS			
	Aman		*Aus*	
	Indrosail	Latisail	Kataktara	Dhariwal
Natural soil	5	15	9	4
Infected soil	7	21	31	8
Water-logged soil	43	38	16	41
Water-logged infected soil ..	85	95	17	50

It will be seen from the table:—

(i) The number of plants infected is greater in water-logged soil than in natural soil.

(ii) The number of infected plants is greater in *Aman* varieties, viz. 'Indrosail', 'Latisail' than in *Aus* varieties, viz. 'Kataktara', 'Dhariwal'.

(iii) Soil inoculated with infected seeds causes a greater incidence in the number of infections in plants than uninoculated soil.

The greater number of infected plants in water-logged soil is probably due to the favourable effect of high moisture content on the germination and growth of *Helminthosporium* although growth of the plants in water-logged soil is much better than in ordinary soil. Different varieties of rice plant were inoculated with *Helminthosporium* on sound tissues of the leaf and the time required for the infection to appear on the spot with slight browning caused in the tissues was noted (Table II).

TABLE II

Time required for infection to appear on leaf

Varieties of Paddy	Time required for infection to appear
Dhariwal	3-4 days.
Bhasamanik	6-7 ,,
Nagra	6-7 ,,
Jhingasali	4-5 ,,
Chinsura	4-5 ,,
Patnai	4-5 ,,
Latisail	4-5 ,,
Tilak Kachuri	4-5 ,,

Certain varieties such as 'Dhariwal' required lesser time for infection than other varieties.

The influence of degree of maturity of the plant on resistance to infection was studied by inoculating two varieties, 'Dhariwal' and 'Kataktara' (*Aus*) with *Helminthosporium* at different stages of maturity. Table III shows the percentage of infections of seedlings on the 7th day.

TABLE III

Percentage infection of rice plant at different stages of maturity

Age of plant	Varieties	P.C. of infection
22 days ..	Dhariwal	70%
	Kataktara	55%
44 „ ..	Dhariwal	55%
	Kataktara	30%
66 „ ..	Dhariwal	10%
	Kataktara	5%

Immature leaves 22 days old are more liable to infection than mature ones. With increasing maturity of the plant there was a decrease in the percentage number of infections on the leaves. The percentage number of infections is greater with 'Dhariwal' variety than with 'Kataktara' variety.

It thus appears that:—

(i) The percentage number of plants infected, and the time required for infection provide a measure of the degree of resistance of the plant, and consequently varieties showing greater number of infections and lesser time required for infection are more susceptible to infection, than varieties showing lesser number of infections and greater time for infection.

(ii) *Aman* varieties are more susceptible to infection than *Aus*. The presence of infecting units or infected seeds in the soil causes a greater incidence of infections in plants than the soil free from infecting units. Young seedlings are more liable to infection than mature plants.

The effect of the nature of inoculation, on the time required for infection and the extent of infection was studied by inoculating 'Bhasamanik' (*Aman*) either on sound leaf and stem or on wounded leaf and stem by using an inoculum of spores in sterile water, protein extract and 0·5% glucose solution. Table IV shows the total number of infections and the time required for the development of infection of 5 mm. size.

It will be seen that:—

(i) The total number of infections is greater on scraped tissues than on sound tissues.

(ii) The total number of infections is greater with inoculum of spores in protein extract and 0·5% glucose than with sterile water.

(iii) The total number of infections is greater on leaf than on stem, the number of infections on scraped tissues being 36 and the number on sound tissues being 21. The time required for infection is greater on sound tissues than on scraped tissues, but time required for infection on stem both on scraped as well as sound tissues varies from 5-6 days, the total number of infections being 2 with protein extract.

TABLE IV

Total number of infections and time required for infection. Twelve points are inoculated in each set

Variety	Parts of plant	Type of inoculum	Nature of inoculation	Time required for infection	Total number of infections
'BHASAMANIK' (*Aman*)	Leaf ..	Sterile water	Sound tissue	6 days	5
			Scraped tissue	3 ,,	12
		Protein extract	Sound tissue	6 ,,	10
			Scraped tissue	3 ,,	12
		0·5% Glucose	Sound tissue	5 ,,	6
			Scraped tissue	3 ,,	12
	Stem ..	Sterile water	Sound tissue	6 ,,	0
			Scraped tissue	6 ,,	0
		Protein extract	Sound tissue	6 ,,	0
			Scraped tissue	5 ,,	2
		0·5% Glucose	Sound tissue	6 ,,	0
			Scraped tissue	6 ,,	0

It appears, therefore, that scraped tissues especially on leaf are more liable to infection than sound tissues, since by scraping or wounding the epidermal tissues, the innermost palisade cells susceptible to infection are exposed to infection by the fungus. It is difficult to explain why leaf is more susceptible to infection than the stem and this difference in degree of resistance is presumably due to the chemical nature of the cell wall capable of resisting the fungal attack (Baruah, 1942). This marked stimulating effect of the addition of protein extract and sugar solution to inoculum of spores on degree of resistance of plant to infection has been studied by examining another variety, 'Chinsura' (*Aman*). Inoculations were made by using an inoculum of spores as used in the previous experiment (Table IV) on sound leaf and stem. Table V shows the time required for infection and the total number of infections.

TABLE V

Total number of infections and time required for infection. Twelve points are inoculated in each set

Variety	Parts of plant	Type of inoculum	Nature of inoculation	Time required for infection	Total number of infections
'CHINSURA' (*Aman*)	Leaf ..	Sterile water	Sound tissue	6 days	1
		Protein extract	Sound tissue	5 ,,	6
		0·5% Glucose	Sound tissue	4 ,,	5
	Stem ..	Sterile water	Sound tissue	6 ,,	2
		Protein extract	Sound tissue	6 ,,	4
		0·5% Glucose	Sound tissue	5 ,,	4

The total number of infections is greater with protein extract and 0·5% glucose than with sterile water, both on stem and on leaf and similarly the time required for infection is less with protein extract and glucose solution than with water. This increase in the degree of susceptibility of plant to infection, i.e. increase in the total number of infections with a corresponding decrease in the time required for infection is probably due to ease of establishment of the fungus caused by enzymes produced by the fungus in the presence of protein extract, since the production of cell-wall-splitting enzymes is promoted by the nitrogen content of the substrate (Baruah, 1942). If, however, the resistance of the epidermis is destroyed by exposing the leaf or stem to chloroform vapour it is possible to induce infection on sound tissues by using an inoculum of spores in water (Table VI). 'Bhasamanik' and 'Chinsura' varieties were exposed to chloroform vapour for half-an-hour and inoculated with *Helminthosporium* by using an inoculum of spores in sterile water and were incubated at 23°–25°C. in moist chambers. Eight points were inoculated in each set.

TABLE VI

Time required for infection and total number of infections

Varieties	Parts of plants	Types of inoculum	Time required for infection		Total number of infections	
			Nature of inoculation			
			Sound tissue	Chloro-formed tissue	Sound tissue	Chloro-formed tissue
'BHASAMANIK' ..	Leaf	Sterile water	6	4	5	8
	Stem	Sterile water	6	6	0	0
'CHINSURA' ..	Leaf	Sterile water	6	5	1	8
	Stem	Sterile water	6	6	2	3

There was an increase in the number of infections on chloroformed tissues than on sound tissues.

Green (1932) has also demonstrated that sound oranges could be infected by *Penicillium digitatum* by subjecting the fruits to chloroform vapour and that the degree of resistance of the fruit to infection decreases by the loss of the semi-permeability of the membrane of the skin. The rapid ease with which *Helminthosporium* spreads in chloroformed tissues indicates that once the resistance of the skin is broken down the underlying cells in the mesophyll being susceptible to attack, cause establishment of the infection and rapid spread of the disease and hence on wounded or chloroformed tissues the number of infections is greater than on sound tissues.

The specificity of parasitism of *H. Oryzae* on rice plant is probably due to the suitability of the composition of the rice plant or rice to the spread of the disease. From studies on the parasitism of disease on a particular host it is evident that for a fungus to parasitize a particular host the conditions must be such as to favour the establishment of the parasite on the host and the spread of the disease on the host.

The effect of the composition of the medium on the rate of growth of *H. Oryzae* was, therefore, studied.

Helminthosporium was grown on various media and the rates of growth were measured (Table VII) according to the methods described by Baruah (1942).

TABLE VII

Linear spread of colony in mm. per five days

Media	Linear spread of colony in mm. per five days
Dox medium ..	85 mm.
Rice plant extract..	82 ,,
Rice root extract ..	71 ,,
Modified Dugger ..	74 ,,

The rate of growth of *Helminthosporium* is highest on Dox medium as well as on rice plant extract medium and lowest on rice root extract. The mycelium produced on rice plant extract was dense and highly coloured black, whereas, on Dox medium mycelial growth was scanty.

The effect of addition of rice plant extract and ash of grass, unhusked rice and rice plant on the growth of the fungus was investigated to find out if the addition of plant extract and ash had any stimulating action on the growth of the fungus.

The addition of small amounts of extract from rice plant and also ash of rice plant, grass and unhusked rice to Dox medium was to increase considerably the rate of growth of the fungus (Table VIII). Ten gms. of each of the materials—rice plant, grass and unhusked rice—are ashed and added to 200 c.c. of Dox medium.

TABLE VIII

Linear spread of colony in mm. per five days

Media	Linear spread of colony in mm. per five days
Dox medium	85 mm.
,, ,, +1% rice plant extract	86 ,,
,, ,, +10% rice plant extract ..	98 ,,
,, ,, +20% rice plant extract ..	93 ,,
,, ,, +312 mg. rice plant ash ..	116 ,,
,, ,, +594 mg. grass ash	106 ,,
,, ,, +508 mg. unhusked rice ash ..	122 ,,

The addition of rice extract increases the rate of growth of *Helminthosporium* from 85 mm. to 98 mm. per five days, whereas, the addition of ash increases it considerably, e.g. from 85 mm. to 122 mm. per five days. The stimulating effect of the addition of plant extract and ash was also observed on *Fusarium* sp., the rate of spread increasing from 60 mm. to 75 mm. per five days. This marked increase in the rate of growth of *H. Oryzae* was also observed with the addition of small amounts of rice plant extract (rice plant extract being centrifuged to remove the fine debris) to Dox medium from which $NaNO_3$ has been eliminated (Table IX).

TABLE IX

Dry weight of mycelium produced per 100 c.c. of medium in nine days

Amount of plant extract in c.c. per 100 c.c. medium	Dry weight of mycelium produced per 100 c.c. medium in nine days
Dox medium (control)	141 mg.
„ „ +1 c.c. plant extract	187 „
„ „ +5 c.c. plant extract	256 „
„ „ +10 c.c. plant extract	261 „
10% plant extract (Rice)	268 „

The stimulating effect of the addition of plant extract as well as plant ash on the growth of the fungus is probably due to (i) nitrogen content of the plant, (ii) carbohydrate content, (iii) the presence of certain trace elements and accessory substances, (iv) hydrogen-ion concentration.

In order to find out which of the factors influences the rate of growth of the fungus in culture, the effect of varying the composition of the medium on the growth of the fungus was studied.

EFFECT OF VARYING NITROGEN CONTENT IN THE MEDIUM

The effect of different sources of nitrogen and of varying the nitrogen content in the medium on the rate of growth of the fungus was studied Asparagine, Peptone, $(NH_4)_2SO_4$, $NaNO_3$, NH_4NO_3 were used as different sources of nitrogen and the rate of growth was determined by estimating the dry weight of the mycelium produced per 100 c.c. of medium in nine days (Table X).

TABLE X

Dry weight of mycelium produced per 100 c.c. medium in nine days

Source of N_2	Amount in gms. per 100 c.c. medium					
	0	0·1	0·25	0·5	1·0	2·0
Asparagine	62 mg.	342 mg.	502 mg.	450 mg.	386 mg.	376 mg.
Peptone	62 „	494 „	884 „	1,002 „	1,280 „	1,360 „
$(NH_4)_2SO_4$	62 „	156 „	175 „	184 „	174 „	148 „
$NaNO_3$	62 „	485 „	604 „	585 „	572 „	452 „
NH_4NO_3	62 „	135 „	149 „	168 „	148 „	123 „

The effect of addition of nitrogenous substances was to increase the rate of growth of the fungus and the effect was most pronounced with Peptone. Higher concentrations of $(NH_4)_2SO_4$, $NaNO_3$, NH_4NO_3, Asparagine inhibit the rate of growth.

EFFECT OF PLANT PROTEIN ON GROWTH RATE

The effect of addition of protein extracted from rice plant was studied on the rate of growth. Protein was extracted according to Osborne's method (1933), dried and added in different concentrations to Dox medium from which $NaNO_3$ had been eliminated. Table XI shows the dry weight of mycelium produced in nine days per 100 c.c. of medium.

TABLE XI

Dry weight of mycelium produced per 100 c.c. medium in nine days

Amount of plant protein in gms. per 100 c.c. medium	Dry weight of mycelium produced per 100 c.c. medium in nine days
0 (control)	126 mgs.
0·005%	151 „
0·05%	190 „
0·1%	276 „
0·5%	304 „

There was an increase in the rate of growth with increase in concentration of protein extract. With 0·5% protein extract the rate of growth was more than double the rate in the control.

EFFECT OF CARBOHYDRATE CONTENT

The effect of addition of Glucose, Sucrose and Starch as different sources of carbohydrate to Dox medium on the rate of growth had been studied (Table XII).

TABLE XII

Dry weight of mycelium produced per 100 c.c. medium in nine days

Amount of carbohydrate in c.c. medium	Dry weight of mycelium produced per 100 c.c. medium in nine days		
	Glucose	Sucrose	Starch
trol) ..	No growth	No growth	No growth
1·0% ..	228 mg.	312 mg.	425 mg.
2·5% ..	505 „	343 „	594 „
5·0% ..	530 „	1,240 „	1,380 „
10·0% ..	1,660 „	2,472 „	885 „
20·0% ..	2,128 „	1,208 „	....

There was no growth in the controls, whereas with the addition of 1·0% Glucose, 1·0% Sucrose and 1·0% Starch there was considerable increase in the rate of growth. There was a gradual increase in the rate of growth with increase in concentration of Glucose, Sucrose and Starch until the optima were reached at 20·0% for Glucose, 10·0% with Sucrose and 5·0% with Starch.

EFFECT OF TRACE ELEMENTS

The marked increase in the rate of growth by the addition of ash is probably due to the presence of trace elements which, in turn, influence considerably the rate of growth of the fungus.

TABLE XIII

Dry weight of mycelium produced per 100 c.c. medium in nine days

Amount of trace elements in gms. per 100 c.c. medium	Dry weight of mycelium produced per 100 c.c. medium in nine days		
	$ZnSO_4$	$MnSO_4$	Lithium citrate
Dox medium (control) ..	362 mg.	362 mg.	362 mg.
0·05%	260 „	564 „	488 „
0·1%	33 „	624 „	481 „
0·5%	24 „	756 „	73 „
1·0%	24 „	654 „	78 „

The addition of Manganese increased the rate of growth considerably, whereas Lithium and Zinc have little or no effect (Table XIII).

Effect of water-soluble Vitamins in rice and Biotin on the rate of growth

The effect of water-soluble fractions of unhusked paddy and rice powder on the rate of growth of *Helminthosporium* was studied. Both unhusked paddy and rice grain were finely powdered and 1 gm. of each was added to 10 c.c. of water and the water extract of each, obtained by filtering after one hour of thorough shaking in the shaker, was added to 100 c.c. of Dox medium. The effect of adding 1·0% Yeast extract was also studied (Table XIV).

Table XIV

Linear spread of colony in mm. per five days

	Dox medium (control)	Dox+10 c.c. unhusked paddy extract	Dox+10 c.c. rice extract	Dox+1·0% Yeast powder
R.G.	62 mm.	100 mm.	95 mm.	70 mm.

There was a marked increase in the rate of growth by the addition of water-soluble fractions of unhusked paddy and rice grain and Yeast powder. This marked increase in the rate of growth is probably due to Biotin and other vitamins present in unhusked paddy or rice grain.

Effect of Hydrogen-ion concentrations

The effect of hydrogen-ion concentration on the rate of growth of the fungus was studied (Table XV). The *p*H optimum for the growth of *Helminthosporium* was from 5·8 to 7·6.

Table XV

Dry weight of mycelium produced per 100 c.c. medium in nine days

*p*H	Dry weight of mycelium produced per 100 c.c. medium in nine days
2·1	No growth
3·6	254 mg.
4·8	291 ,,
5·8	314 ,,
6·3	302 ,,
7·6	277 ,,
8·5	252 ,,
9·8	214 ,,

The results of the foregoing experiments may be briefly summarized as follows:—

(i) The rate of growth of *H. Oryzae* is highest in rice plant extract and rice medium and on synthetic media to which small amounts of plant extract are added.

(ii) The growth-stimulating effect of rice plant or rice extract is not due to a single constituent but to several factors.

(iii) The rate of growth is influenced by nitrogen, carbohydrate, trace elements and water-soluble vitamins. With the addition of 0·5% protein extract the

rate of growth was more than double the rate in the control. There is considerable increase in the rate of growth of *Helminthosporium* with the addition of Glucose, Sucrose and Starch, rice plant ash, Manganese. The *p*H optimum for growth of the fungus was from 5·8 to 7·6 in culture.

The marked suitability of rice plant or rice as a medium for growth appears, therefore, to be due to the presence of nitrogen, especially proteins, carbohydrate, trace elements and water-soluble vitamins and hydrogen-ion concentration.

How the breakdown of tissues is affected by enzymes secreted by *Helminthosporium* during its growth has been studied by determining the amount of reducing sugar formed during hydrolysis of rice and paddy by *Helminthosporium* extract. Enzymes were extracted by method described by Baruah (1942), and reducing sugar formed was estimated by Lane-Eynon General Volumetric method and Scales method Tentative (1935). *Fusarium* extract was also tried. Table XVI shows the amount of reducing sugar formed after 24, 48, 72 hours during hydrolysis of rice grain and paddy seeds powder (powders being obtained by grinding unhusked and husked rice) by *Helminthosporium* and *Fusarium* extract. For experimental purposes 1 gm. of cell wall material prepared from unhusked or husked rice was treated with 100 c.c. of 2% *Helminthosporium* extract at *p*H 5·2, and the reaction mixture was incubated at 28°–30°C.

TABLE XVI

Amount of reducing sugar formed after hydrolysis by Helminthosporium and Fusarium extract at 28°–30°C.

Reaction mixture	0 (control)	24 hrs.	48 hrs.	72 hrs.
R.+H. extract	nil	0·0958 gm.	0·2128 gm.	0·3290 gm.
P.+H. „	„	0·0918 „	0·1091 „	0·1662 „
R.+F. „	„	0·0891 „	0·1106 „	0·1573 „
P.+F. „	„	0·0738 „	0·0917 „	0·1120 „

R. = Rice grain powder. P. = Paddy powder. H. = Helminthosporium. F. = Fusarium.

It will be seen that:—

(i) The amount of reducing sugar formed is greater with *Helminthosporium* extract than with *Fusarium* extract; there is a gradual increase in the amount of sugar formed, the highest being reached at 72 hours.

(ii) The amount of reducing sugar formed is greater with rice than with unhusked rice.

H. Oryzae extract on being tested on bran (rice), leaf blade or stem, apple, orange, lemon skin shows no lamellase action (Baruah, 1942), and it is thus possible that besides carbohydrase action, *Helminthosporium* extract has little or no pectinase action.

CONTROL OF WASTAGE IN FIELD AND STORAGE

The control of wastage of paddy in the field presents a difficult problem since the fungus is capable of living as a semi-saprophyte in the soil and can also live over in the winter on other grasses. Methods had therefore always been looked for; any method that would reduce incidence of infection of the paddy plants in the field and of grains or paddy seeds in storage, would be of advantage in preservation of such an important

staple foodstuff. Obviously, one of the methods likely to reduce infection to a marked degree should be to remove infected plants and destroy them by burning, thereby reducing the presence of the number of infecting units, and also use of resistant varieties of paddy. Ocfemia (1924) has shown in Philippines that different varieties of rice exhibit different degrees of infection. From the evidence given above it has been found that *Aman* and *Aus* varieties of paddy show different degrees of resistance to infection. The other method would be to treat seeds, disease being seed-borne, with chemicals having fungicidal properties, thereby reducing the number of infecting units.

Hundred seeds were treated differently by various chemicals and sown in pots. Table XVII shows the number of healthy seedlings germinated from seeds treated with chemicals.

TABLE XVII

Number of healthy seedlings germinated after treatment

Nature of treatments	NUMBER OF PLANTS GERMINATED			
	'Dhariwal'		'Kataktara'	
	Ordinary soil	Water-logged soil	Ordinary soil	Water-logged soil
Control	95	59	72	81
Hot water	89	71	83	79
Hot water + 10% soap	89	56	30	30
$CuSO_4$, 2·5%	83	67	82	79
Formaldehyde 2·0%	96	78	81	82
Salicylic acid 0·2%	37	65	69	86

Formaldehyde shows the largest number of healthy plants germinating from treated seeds, but it is difficult to explain why the number of healthy seedlings germinating from treated seeds bears no relation to the fungicidal effect of the chemicals used. Ocfemia (1924) has also found that the number of blighted seedlings is always the same in untreated seeds as in seeds treated with 1 : 1000 $HgCl_2$.

Paddy seeds were treated with different chemicals and inoculated with paddy seeds infected with *Helminthosporium* and stored in moist chamber at 23°–25°C., results are shown in the Table XVIII.

TABLE XVIII

Extent of infection of paddy seeds treated with chemicals

Type of fungicides	Nature of treatment	P.C. of infection
Formaldehyde	5% for ½ hour	nil
H_2O_2	5% for ½ hour	++
Hot water	50°–55°C. for ½ hour	+++
Hot water + soapchips ..	50°–55°C. for ½ hour	++++
Benzaldehyde	5% for ½ hour	nil
Diphenylamine	5% for ½ hour	nil

+ = 25%, ++ = 50%, +++ = 75%, ++++ = 100%.

There was no infection of paddy seeds treated with Formaldehyde, Benzaldehyde and Diphenylamine, whereas with hot water and hot water and soap treatments considerable infection was observed. By treatment with H_2O_2 the extent of infection was considerably less than with hot water and hot water and soap.

The effect of Diphenyl on the rate of growth of the fungus was investigated and it was found that the addition of 0·005% Diphenyl to Dox medium inhibits completely the growth of the fungus (Table XIX).

TABLE XIX

Linear spread of colony in mm. per five days

Amount of Diphenyl in gms. per 100 c.c. medium	Linear spread of colony in mm. per five days
Dox medium (control)	85 mm.
" " +0·005%	nil
" " +0·01%	"
" " +0·05%	"
" " +0·1%	"
" " +0·5%	"
" " +1·0%	"

Tomkins (1935) first showed that Diphenyl inhibits the green mold rot of orange and that wrappers impregnated with Diphenyl reduce not only the number of rotting fruits but also the spore formation accompanying rotting.

The effect on the extent of infection of treatment of paddy seeds with different concentrations of Diphenyl was investigated. 'Dhariwal' (*Aus*), 'Nagra' (*Aman*), 'Boro' paddy seeds were used; seeds after being treated with Diphenyl were inoculated with infected paddy seeds and stored in moist chambers at 23°–25°C. for seven days. Table XX shows the extent of infection of different varieties of paddy seeds at different concentrations of Diphenyl.

TABLE XX

Extent of infection of paddy seeds at different concentrations of Diphenyl after seven days

Varieties	Control without treatment	0·005%		0·01%		0·05%		0·1%		0·5%		1·0%	
		TC	TI	TC	TI	TC	TI	TC	TI	TC	TI	TC	TI
'Dhariwal'	++++	−	++	−	+	−	−	−	−	−	−	−	−
'Nagra'	++++	−	+++	−	+++	−	−	−	−	−	−	−	−
'Boro'	++	−	+	−	+	−	+	−	−	−	−	−	−

TC = treated control, TI = treated inoculated with infected seeds.

Infection was highest in the control without treatment with Diphenyl. With the addition of 0·005% and 0·01% Diphenyl there was a decrease in the extent of infection. This decrease was considerably less in 'Boro' and 'Dhariwal' varieties than in 'Nagra' variety. With further increase in concentrations of Diphenyl up to 0·05%, there was no infection.

EFFECT OF WRAPPERS AND GUNNY BAGS IMPREGNATED WITH DIPHENYL ON INFECTION

Cellophane papers treated with 0·2% Diphenyl were used for wrapping paddy seeds of 'Dhariwal', 'Nagra' and 'Boro' varieties. Controls were set up by using ordinary wrappers. Paddy seeds were inoculated with infected grains and wrapped with treated wrappers and ordinary wrappers and stored at 23°–25°C.; results after the 4th day are shown in the Table XXI.

TABLE XXI

Extent of infection of paddy seeds after using impregnated wrappers

Varieties	Ordinary wrappers (control)	Impregnated wrappers
'Dhariwal'	++	+
'Nagra'	++	+
'Boro'	+	nil

The use of impregnated wrappers reduces considerably the extent of infection of 'Dhariwal' and 'Nagra' varieties, whereas, there was no infection in 'Boro' variety. The effect of storing paddy seeds in gunny bags treated with 0·2% Diphenyl and in ordinary bags was investigated. Paddy seeds were inoculated with infected seeds and stored in treated bags and in ordinary bags at 28°–30°C. An examination after one month of the bags showed there was no infection of paddy seeds stored in treated bags whereas there was some infection of paddy seeds stored in ordinary bags.

Of the chemicals tried it appears that Diphenyl applied either in the form of vapour in impregnated wrappers or by directly treating the paddy seeds reduces the infection of rice by *Helminthosporium*.

The effect of humidity on the extent of infection of rice was also studied, since humidity is another important factor influencing the storage life. Rice was stored at different concentrations of relative humidities in storage chambers at 28°–30°C., and the extent of infection after different periods was observed (Table XXII).

TABLE XXII

Extent of infection of rice stored at different humidities

Relative humidity %	4th day	12th day	After three months
100 ..	++	+++	++++
97·5 ..	++	+++	++++
95·1 ..	+	++	++++
92·6 ..	−	+	+++
90·2 ..	−	+	++
87·7 ..	−	+	++
85·1 ..	−	+	+
82·2 ..	−	−	+
80·0 ..	−	−	+
76·0 ..	−	−	+

+ = 25%, ++ = 50%, +++ = 75%, ++++ = 100%.

The extent of infection was highest at 100% R.H. and with a corresponding decrease in humidity there was a decrease in the extent of infection, until at 82·2%, 80·0% and

76·0% R.H. there was no infection on the 12th day. There was slight infection at 76·0% R.H. after three months.

Effect of humidity and Diphenyl on storage of rice

The effect of reducing humidity of the storage atmosphere in the presence of Diphenyl on the extent of infection was investigated. Rice grains were stored at different humidities at 28°–30°C. Diphenyl was introduced by putting a few crystals (0·2 gm.) inside the storage chamber the volume of which is approximately 350 c.c. (Table XXIII).

Table XXIII

Extent of infection of rice stored at different humidities in presence of Diphenyl

Relative humidity %	4th day	12th day	After three months
100 ..	+	+++	++++
97·5 ..	+	+	+++
95·1 ..	−	−	−
92·6 ..	−	−	−
90·2 ..	−	−	−
87·7 ..	−	−	−
85·1 ..	−	−	−
82·2 ..	−	−	−
80·0 ..	−	−	−
76·0 ..	−	−	−

There was no infection on 4th and 12th day by reducing humidity to 95·1% in the presence of Diphenyl, whereas there was infection of grains at 87·7% R.H. without Diphenyl. The effect of control of humidity to 85·1% R.H. was to reduce infection, whereas in presence of Diphenyl reducing humidity to 95·1% was sufficient to check completely infection of rice grains. There was no infection even at 95·1% R.H. after three months in presence of Diphenyl.

Conclusions

The conclusions reached above concerning the parasitism of rice by *Helminthosporium Oryzae* Breda de Haan and its control are as follows:—

Helminthosporium causes wastage of paddy in the field and rice in storage. The extent of infection depends upon the type of seeds used, presence of spores in soil and on the ability of the fungus to grow on other members of Graminae and climatic conditions. The greater percentage of infections of rice plants in infected soil than in ordinary soil is probably due to the presence of the infecting units attacking the seedlings, which in their turn are more liable to infection than mature ones. The change in the physical conditions of the soil by increasing the moisture content by water-logging causes a greater percentage of infections than ordinary soil because high moisture is favourable for the germination and growth of the fungus. Chiappelli (1929) has also found that the attacks of *H. Oryzae* in rice plants are favoured by water-logging. Ocfemia (1924) suggests that infection can be prevented by submerging the soil in water to a depth of about 10 cm. provided temperature of water is maintained at 24°–28°C.

It is possible to attribute the blighting of the seedlings to attack of the root and shoot systems by the fungus and the marked severity of lesions on the plant, e.g. stem, leaf and panicle in the field to infection by *Helminthosporium*. Evidence is given that sound

tissues are infected by *Helminthosporium* causing brown lesions in four-five days whereas wounded or scraped tissues are infected in three days on leaf and in five-six days on stem. The number of infections obtained on sound and scraped tissues by using an inoculum of spores in protein etxract, 0·5% glucose solution and sterile water shows that given an inoculum of spores in protein extract and 0·5% glucose solution, the number of infections is greater than with sterile water. Since in any instance of parasitism of a certain host by a particular parasite, it is the conditions in the plant which determine the degree of susceptibility of the plant to disease and the capacity of the fungus to grow in the host and cause breakdown of the host tissues. The establishment of infection on wounded tissues is possible, because by removal of the cuticular and epidermal resistance of the skin, by wounding or by chloroform vapour, the inner palisade cells susceptible to infection are exposed to attack, and hence infection is easily established; but on sound tissues it is possible that the fungus grows on skin, transpiration water and moisture being sufficient to cause the germination of the conidia and the germ tube penetrates directly through the stomata (Nisikado and Miyake, 1922). Once the fungus establishes itself on the plant, paddy seeds, the rate of spread of the fungus is influenced by the nutritive composition of the host. It has been demonstrated that the addition of proteins, extracted from rice plants, nitrogenous substances, carbohydrates, trace elements chiefly Manganese and accessory substances of the nature of Vitamins B_1, Riboflavin (G_1) has a marked stimulating effect on the rate of growth of the fungus, and that *p*H optimum for the growth of the fungus is from 5·8 to 7·6. It is thus possible that the spread of *Helminthosporium* on the plant or on rice is influenced not by one factor alone but by several factors, e.g. nitrogen, carbohydrate, trace elements, accessory substances and hydrogen-ion concentration.

Evidence is also given of the activity of enzymes produced by the fungus; it is found that the enzymes have no action on pectic constituents of the cell wall but are capable of hydrolyzing the starch molecule to form reducing sugar. The extent of hydrolysis of the unhusked rice and husked rice by the fungus extract indicates that the enzymes attack the starch constituents of the endosperm, but have slight or no action on unhusked paddy or on husk alone.

The control of wastage of paddy in the field resolves itself into removing the source of infection in the soil by irrigation and burning grasses and other infected materials and use of healthy seeds, because treatment of infected paddy seeds with fungicides does not kill the spores or mycelium lying dormant in between the husks inside the ridges (Ocfemia, 1924). The use of Diphenyl, however, presents certain possibilities because both Diphenyl treatment and Diphenyl vapour inhibit completely the growth of the fungus. Use of Diphenyl treated wrappers or bags checks infection and even the introduction of Diphenyl checks infection of paddy seeds stored at high humidities. Reducing humidity alone to 82·2% R.H. checks infection but Diphenyl checks infection at 95·1% R.H. By application of this method of treatment of Diphenyl on paddy seeds before sowing and by using treated gunny bags or storing in presence of Diphenyl at humidities below 95·1% R.H. it is possible to reduce the extent of wastage in the field and also check decomposition of rice in storage.

SUMMARY

1. *Helminthosporium Oryzae* Breda de Haan causes wastage of paddy in the field and rice in storage, the incidence of disease in the field being influenced by the type of seeds used, presence of infecting units in soil and grasses and climatic conditions.

2. The extent of infection of paddy plants in the field varies with the degree of maturity of the plant and the variety used.

3. The rate of growth of *H. Oryzae* is influenced by the composition of the medium; the addition of extracts from rice plant, protein extract, ash, stimulates the rate of growth of the fungus. The presence of nitrogenous compounds, carbohydrates, trace elements and other accessory factors of the nature of water-soluble vitamins of rice grains increases the rate of growth to the same order as that obtained by growing the fungus alone on rice plant medium, rice grain or paddy seeds medium.

4. The rate of breakdown of paddy seeds and rice grains by enzymes extracted from the fungus shows that the extract breaks down starch completely to sugar but has no action on the pectic constituents of the cell wall.

5. The wastage of paddy in the field and in storage can be reduced successfully by methods of deep irrigation and removal of alternate hosts and by treating paddy seeds with Diphenyl or storing in presence of Diphenyl vapour.

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VI. STUDY OF MULTIPLE IONIZATION TRACK SPECTRA ON PHOTOGRAPHIC PLATES EXPOSED TO COSMIC RAYS AT DIFFERENT ALTITUDES

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INTRODUCTION

§1. It is now a well-accepted fact that photographic plates are one of the very useful tools for the study of ionization tracks due to charged particles. Several investigators have reported the presence of α-particles and proton tracks in photographic plates, but none of them have found any evidence regarding β-particle tracks.

We have also published several studies on single tracks due to ionizing particles observed in photographic plates which were exposed to cosmic rays at altitudes up to 14,500 ft.

In a joint note by Bose and Chowdhuri (1942) published in *Nature* and in a recent paper published by the present author (1944) in *Indian J. of Physics*, a method for the determination of the mass of these ionizing particles, responsible for single tracks in photographic plates, was described. The mass values obtained were found to be within the usual accepted range of values for mesons. In these communications attention was also drawn to the presence in the photographic plates of many multiple tracks. By multiple tracks we mean two or more than two tracks radiating from a single silver grain.

Some of the characteristics of these multiple tracks were described in a joint note published by Bose, Chowdhuri and Sinha (1944) in *Phys. Rev.*, and they were compared with the results to be expected on a recently published theory of the energy distribution in meson spectra proposed by Hamilton, Heitler and Peng (1943).

Multiple nuclear disintegrations by cosmic rays have been observed in cloud chambers. Brode and Starr (1938) reported that at sea-level out of 20,500 counter-tripped cloud chamber photographs of cosmic rays, 10 tracks are due to nuclear disintegration.

Anderson (1936) found at Pike's Peak (4,300 m.) that out of 9,188 exposures with Wilson Chamber only 123 heavily ionizing tracks were observed.

Recently several observers have obtained photographs of meson multiples in Wilson Chamber.

A meson pair was obtained by Herzog (1941) at an elevation of 29,000 ft. in Wilson Chamber. At an altitude greater than 15,000 ft., he obtained several photographs containing two to four slow mesons. A meson pair was obtained which was stopped in 0·32 cm. copper. Assuming these particles have mass 200 times electron mass their energies are found to be of the order of 25 MeV.

Hughes (1941) obtained a photograph of slow meson pair at Peru (15,500 ft.) with kinetic energy $\sim$ 5 MeV. Their masses are found to be 189 $\pm$ 26,180 respectively.

Wollan (1941) at an altitude of 15,500 ft. reported a number of photographs containing two particles diverging from a point, obtained from 1,500 counter-controlled Wilson Chamber photographs, with 15 cm. Pb above the chamber. Further it was observed that these particles traversed a thickness of 2 cm. of lead without showing any multiplication effect, from which he concludes that they are meson pairs.

Powell (1941) has obtained a photograph of a pair of meson tracks produced by non-ionizing particles which penetrate several lead plates each 1 cm. thick.

Recently M. Sinha (1943) has reported a few meson multiples in an investigation with counter-controlled Wilson Chamber at Bose Institute, Calcutta.

All these investigations excepting the last-mentioned one were carried out at high altitudes. Thus even at high altitudes very few multiple tracks were observed by cloud chamber technique. For the study of these rare events photographic plates represent a very useful device. A comparison with a Wilson Chamber shows that while the latter has a fairly large recording volume containing low density matter and a small sensitive time of the order of 0·1 sec., a photographic plate has a small recording volume of large density, and of indefinitely large recording time, e.g. up to one year.

The general view held by previous workers on the nature of multiple tracks observed in photographic plates is that these are due to protons resulting from some close interaction between the cosmic rays and the nuclear particles present in the photographic plates.

Blau and Wambacher (1937) reported the simultaneous generation of several particles from a common centre in their Hafelkar plates. From a comparison of the mean grain spacings (m.g.s.) along these tracks with those observed in proton tracks of energy up to 10 MeV, ejected from paraffin by Beryllium neutrons, they concluded that the former are also produced by protons.

Schopper and Schopper (1939) sent Agfa K-plates to the stratosphere and obtained some very interesting results. Their investigations reveal the presence of several star-like tracks in these plates which they interpreted as either due to protons or α-particles or some other multiple charged particles.

Investigations were also carried out by some Russian workers, Zhadnov *et al.* (1944). According to them these tracks can be classified in two groups: in which (*a*) the particles emerge in random directions from a common centre; (*b*) particles emerge from a common centre but are confined within a small solid angle. This last-mentioned group was designated as proton showers.

Several other investigators have also obtained similar tracks on photographic plates exposed to cosmic rays at high altitudes and the majority of them have interpreted these multiple tracks as due to protons.

Shapiro (1941) from a statistical investigation of 365 tracks in 142 stars, produced by cosmic rays in a photographic plate, estimated that more than 90% of the tracks were produced by protons and the remaining 10% were probably due to α-particles of energy < 9 MeV.

Turning to our own investigations, it was observed by Bose and Chowdhuri in a Sandakphu (12,000 ft.) plate that several small angled double tracks were present. In a short note in *Nature* (1940) they interpreted these as due to emission of meson pairs. This interpretation was further supported by the estimation of the average mass of single ionizing particles, according to a method developed by them (1942); the latter was found to be of the order of 200 m_0. Similar results were obtained for the masses of the particles producing the pair tracks.

From these results the conclusion was drawn that these Ilford Halftone plates not only record tracks of α-particles and protons but are sensitive enough to record tracks produced by mesons; and further that the majority of tracks in these stars are due to multiple generation of mesons.

In the present paper a detailed account is given of the distribution of such multiple tracks in photographic plates placed under different absorbing layers at different altitudes,

Pharijong (14,500 ft.), Sandakphu (12,000 ft.) and Darjeeling (7,000 ft.). This paper includes also an interpretation of the nature of the primary beam responsible for these multiple tracks. In §2 an account is given of the emission of different kinds of particles, which have been considered to be theoretically possible, when atomic nuclei are struck by fast particles of protonic nature and of energy ~10 MeV upwards. This will provide a suitable background for the interpretation of our experimental results given in §3 on the frequency of distribution of stars of different multiplicities appearing in photographic plates exposed to cosmic rays. The results obtained are discussed in light of the theory of the origin of cosmic ray meson spectra, found in different altitudes, proposed by Hamilton, Heitler and Peng (1943).

In §4, the absorption of primary particles, responsible for multiple tracks, in different substances is determined and compared with the results to be expected according to the above-mentioned theory. In §5, general conclusions and summary are given.

§2. In theories of nuclear disintegration, the incident particles used have energies of the order of 1 to 10 MeV. According to the theory proposed (Bethe, 1937) for such processes by Bohr, a nucleus 'A', when struck by a fast particle P, gives rise temporarily to a compound nucleus 'C', which will exist in a quasistationary state for a time large compared to the characteristic nuclear time which is of the order of 10^{-23} sec. The latter will be in a state of excitation, in which the energy of the incident particle remains divided between the constituent nuclear particles, till some such time when the energy is again concentrated in one or more particles, of amount sufficient to overcome the potential wall; these particles will be then emitted.

$$A+P\rightarrow C\rightarrow B+Q \quad .. \quad .. \quad .. \quad .. \quad .. \quad (1)$$

where P is the incident and Q the emitted particle. We can consider the emission of particles from the nucleus as analogous to evaporation due to rise of nuclear temperature by absorption of the energy of the incident particle. The energy relation is

$$W_A+W_P+E_P\rightarrow W_B+W_Q+E_Q \quad .. \quad .. \quad .. \quad .. \quad (2)$$

when W_A denotes the internal energy of nucleus 'A' and W_P the internal energy and E_P the kinetic energy of the incident particle, etc.

For nuclear evaporation process two models have been proposed:

(*a*) The nucleus consists of free individual particles with very little interaction between them, so that the total energy of the nucleus is equal to the sum of the energies of the individual particles. The nucleus is then comparable to a gas.

The relation between the temperature and energy of a nucleus is assumed to be of the form

$$U=\alpha T^n \quad .. \quad .. \quad .. \quad .. \quad .. \quad (3)$$

For the gas model, $n=2$; $T=(U/\alpha)^{\frac{1}{2}}=(aU)^{\frac{1}{2}}$, where $a=\cdot 05$ to $\cdot 2$ MeV. For heavy nuclei, the binding energy is of the order of 2×10^9 eV. We can use this formula to explain the emission of particles due to cosmic rays of energy 10^8 eV to 10^9 eV, e.g. for U = 100 to 500 MeV, T ~ 2–10 MeV. As long as the temperature is high, it is to be expected that most of the particles will leave the nucleus with energy of the order of T. Heitler showed that the total number of particles emitted will be given by U/T; for U = 100 MeV, 20 particles will be emitted of which half will be probably neutrons. It is reasonable to expect the emission of 5–10 particles, which is in agreement with Blau and Wambacher's observations.

(*b*) The above model is not, however, in agreement with the known properties of the nucleus, e.g. of its stability, mass defect, etc. It has been replaced by the liquid drop model of Bohr and others. Here the interaction between the nuclear particles is large compared to their kinetic energies. The normal modes of vibration of the constituent particles in the liquid drop can be determined, and to each mode of vibration an excitation energy as given by Planck's formula can be assigned. Summing up we can express the total excitation energy as function of a temperature T introduced in Planck's formula. It is found that in (3) $n = 7/3$ for low temperatures, and equal to 4 for high temperatures. Since the theory has been developed to account for nuclear disintegration, the excitation energy is taken as small compared to the total nuclear binding energy (e.g. 10 MeV as compared to 10^8 MeV); the resulting rise of temperature is rather low.

We can find the probability for the emission of a particle P of energy E from a compound nucleus C in a state of excitation by which a residual nucleus is left with energy W_A. We assume the excitation energy of the residual nucleus W_{A_0}, when $E = 0$, is large compared to E and its temperature is T. It is then found that the probability distribution function of the emitted particles is

$$W^{C}_{AP} . dE \sim e^{-E/T} . \quad E . dE \qquad .. \quad .. \quad .. \quad .. \quad (4)$$

similar to Maxwell's distribution law. The most probable energy of emission is $E = T$. If after emission of the first particle the residual nucleus has still a high energy of excitation, then a second and possible a third particle will be emitted with lower kinetic energies. For the emission of charged particles we have to multiply the right side of equation (4) with the penetrability factor $P = e^{2g\gamma(E/B)}$; where B is the height of the potential barrier and g is a constant and γ is a function of $\frac{E}{B}$. For $E \gg B$, $P \to 1$. For example, in the case of Hg_{200} the height of B is 9·6 MeV and W_{A_0} is 20 MeV; the most probable energy of the outgoing particles is about the height of the potential barrier, while emission of particles of higher and lower energies becomes less probable.

The theory has not been developed for the case where the excitation is $>10^8$ eV where another order of reaction becomes effective: (i) the cross-section for the absorption of primary proton by nucleus decreases very rapidly with the ratio of binding energy to primary energy; (ii) also no consideration has been given to the nature of the short range forces between the nucleons present in a nucleus. According to Yuwaka the latter is deducible from a potential energy function $\phi = \frac{g}{r} e^{-\lambda r}$; the interaction between the meson field and a nuclear particle can then be expressed by two constants g and λ of which g is of order of 5 electron charge, and the rest mass of the interacting particle is given by

$$\mu = \frac{\lambda h}{2\pi c} \sim 150\, m_0.$$

In the theory as originally developed by Yuwaka, the meson field was of a scalar character, and g is the coupling constant between the meson field and the proton particle. In a recent development of the meson field theory, by Möller and Rosenfeld (1941) and which forms the basis of the theory of cosmic ray mesons developed by Hamilton, Heitler and Peng (1943), there are three kinds of meson fields, the longitudinal, transverse and pseudoscalar, with the coupling constants g, f and f' respectively, such that in the natural system of meson units in which $h = c = \mu = 1$; $f^2 = f'^2 = \cdot 013$ and $g^2 = \cdot 054$. In this

system all cross-sections are measured in units of $\left(\frac{h}{2\pi\mu c}\right)^2 = 4{\cdot}3\times10^{-26}$ cm.^{2} Usually the longitudinal field is ignored. According to the theory proposed by Heitler *et al.* (1944, 1945) each elementary particle called nucleon (proton/neutron) carries a short range nuclear meson field; the interaction between a meson and a nuclear particle is a strong one and becomes increasingly stronger at higher energies. If a proton moves with an energy $E > M$ the proton mass, its field is equivalent to a beam of free mesons of various energies ranging from 1 to E. The equivalent energy spectrum of these virtual mesons is given by $q(\epsilon)d\epsilon$. One of these virtual mesons may be scattered in the field of a nucleon at rest, the scattered meson having an energy ϵ' the balance $\epsilon-\epsilon'$ being transferred to the nucleon at rest.

The cross-section for meson production of energy $\epsilon' d\epsilon'$ is then

$$\phi(\epsilon')d\epsilon' = d\epsilon' \int_{\epsilon'} Q(\epsilon, \epsilon')q(\epsilon)d\epsilon$$

where $Q(\epsilon, \epsilon')$ is the cross-section for scattering of the mesons by the nucleon at rest, and the integration is extended over all virtual mesons. The formula holds when $\epsilon \gg 1$, $E \gg M$ and $\epsilon' \ll E$. A similar contribution will arise from the disturbance which the fast nucleon receives from the nucleon at rest, i.e. if the former is deflected from its straight path it will also emit mesons. Under some simplifying conditions the energy loss of the proton is found to be $-dE/dx = 43 \log 0{\cdot}3E$. It is one hundred times larger than the energy loss of protons due to ionization. Thus a proton of energy 3×10^9 eV loses in one cm. path in lead 2×10^9 eV of its energy. The theory does not, however, tell us how quickly a proton loses its energy after being slowed down to an energy E of the order of M. Although meson production is then negligible compared to its rate of production at high energies, still its energy loss is much greater than that due to ionization. A rough calculation shows that energy loss is then about ten times the ionization loss.

So far we have assumed that fast protons produce single mesons only during the process of scattering. Heitler and Peng (1942) have shown that not only are single mesons produced in succession by scattering of fast protons, but several mesons can be produced in one elementary act. The rate of occurrence of the event can be obtained by multiplying the equivalent meson spectra of a fast proton by the cross-section for splitting up of a meson into several mesons instead of by the simple scattering cross-section. This splitting up process has been calculated for the case when all energies concerned are smaller than M. It is found that the splitting up cross-section of multiple process is always less than one-tenth of the ordinary scattering cross-section.

We shall now consider what happens to the energy $\epsilon-\epsilon'$ transferred to the stationary nucleon, when a virtual meson of energy ϵ is scattered by it. Two cases may be distinguished: (*a*) when $\epsilon-\epsilon' > 10^8$ eV, that is, the period of collision $\tau \ll T$, the natural frequency of the recoiling nucleon bound inside a nucleus; the latter will then behave as a free nucleon, and will be able to produce fresh mesons by further collisions with stationary nucleons. This will give rise according to Heitler *et al.* to a cascade production of mesons; (*b*) when $\epsilon-\epsilon' \lesssim 10^8$ eV, i.e. when $\tau > T$; the process is adiabatic and the energy $\epsilon-\epsilon'$ can be absorbed by the nucleus as a whole and the average amount absorbed will be proportional to 1/M, where M is the nuclear mass. The absorbed energy, which is $\sim10^6$ eV, will raise the temperature of the nucleus sufficiently to induce emission by evaporation of one or more nuclear particles, which will be emitted in random directions.

Thus we can expect the following processes to occur during the collision of fast proton-like particles with atomic nuclei.

A1. When the energy of the primary particle is $>10^6$ but does not exceed 10^8 eV, the particle will be captured by the nucleus resulting in the formation of a compound nucleus in a state of excitation represented by an increase in the nuclear temperature. One or more heavy particles will be emitted from the nucleus in more or less random directions.

In Wilson Chamber photographs as well as in photographic plates exposed to cosmic radiations, such emissions of heavy nuclear particles have been observed in small numbers.

A2. It can also happen for large particle velocities, that the primary particle instead of being absorbed by the nucleus, transfers its momentum directly to one or more nucleons in the latter, which are then emitted in more or less forward directions. Some of the photographic tracks of cosmic ray particles, obtained by the Russian workers Zhadnov, Perfilov, Daisenrod (1944) in which the emitted particles lie within a small solid cone, belong to this class.

B. When the energy of the primary particles $>10^8$ eV, single or multiple meson emission will occur; when the struck nucleus is heavy, such meson emission can be accompanied by the simultaneous emission of heavy particles. Such mixed multiple starlike tracks have been observed in our plates kept exposed to cosmic rays at high altitudes. The energy involved in the emission of heavy particles will, if our hypothesis is correct, be of the order of 10^6 eV; they will produce heavy ionization tracks in our emulsion with mean grain spacing $<2{\cdot}4\times10^{-4}$ cm. So when calculating the energy E of the primary particle producing n meson tracks in a single act we can always neglect the energy utilized in the production of heavy particles like proton. Thus if in a multiple of m tracks, p have mean grain spacing along their tracks $<2{\cdot}4\times10^{-4}$ cm., the actual meson number in it will be $n = m-p$.

Besides protons producing meson multiples, high energy mesons can also produce meson multiples by process of scattering in the field of nucleon. In addition, energetic light quanta have a small probability of producing mesons by the process $h\nu+\mathrm{P}\rightarrow\mathrm{N}+\mu^+$; instead of producing electron pairs. The cross-section for this process has been found by Hamilton and Peng (1944) to be $\sim 2\pi ef/\epsilon^2$, for pseudoscalar mesons for $\epsilon < \mathrm{M}$.

We shall in course of the discussion of our experimental results show how far the latter are in agreement with the predictions of the theory of Heitler *et al.* Considering the large production cross-section for mesons by fast protons as given by Heitler *et al.*, Janossy (1943) proposes a slight modification in the above theory.

According to the latter, due to large scattering cross-section, a fast nucleon can be expected to suffer collision several times while crossing an atomic nucleus. Thus it can produce a group of mesons in a collision with a single nucleus instead of producing them one by one in succession. According to this hypothesis the heavier the atomic weight of the traversing medium the larger will be the multiplicity of the meson shower; further, it is assumed that nuclear particles are independent of one another, which is correct for the case of emission of fast mesons but in the case of slow mesons interference effect due to the constituent nuclear particles will be effective.

Experimental Results

§3. Ilford Halftone photographic plates with emulsion of thickness 70×10^{-4} cm. were kept for several months at different altitudes, viz. Darjeeling (7,000 ft.), Sandakphu (12,000 ft.) and Pharijong (14,500 ft.). A number of plates were left directly exposed to

radiation while others were kept under different absorbing substances such as water, paraffin and lead. The thickness of the absorbing water column and paraffin blocks was always taken equal to be 20 cm., but lead blocks of six different thicknesses varying from 0·5 cm. to 5 cm. were used as absorbers. The investigations with photographic plates placed under air only were repeated twice. The plates used in the two occasions were not from the same batch. After development several single and a number of multiple tracks were observed. Here in this paper we shall deal only with multiple tracks.

TABLE I

Frequency of multiple tracks						Place	Absorbing substance	Time of exposure	Area examined
2	3	4	5	6	7				
16	20	18	4	2	1	Sandakphu Plate I	Air	150 days	1·2 cm.²
4	14	10	4	0	0	,, ,, II	,,	163 ,,	1 ,,
0	9	7	11	1	0	Pharijong ,, I	2½ ft. mud and wood	97 ,,	2·5 ,,
3	18	13	9	0	2	,, ,, II	,, ,, ,,	209 ,,	1 ,,
5	19	10	3	2	0	Darjeeling ,, I	Air	150 ,,	1 ,,
2	7	5	1	0	0	,, ,, II	,,	180 ,,	1 ,,
8	8	5	4	0	0	Sandakphu	Water (20 cm.)	202 ,,	1 ,,
4	10	9	2	0	0	,,	Paraffin (20 cm.)	163 ,,	2·0 ,,
2	4	6	5	0	0	Pharijong	,, (20 cm.)	209 ,,	1·0 ,,
5	15	18	7	2	0	Sandakphu	Lead (·5 cm.)	163 ,,	1·5 ,,
8	22	19	8	1	0	,,	,, (1·0 cm.)	163 ,,	1·5 ,,
14	33	21	15	1	0	,,	,, (1·5 cm.)	163 ,,	1·5 ,,
9	21	18	5	1	0	,,	,, (2·0 cm.)	163 ,,	1·5 ,,
15	18	12	4	1	0	,,	,, (3·0 cm.)	163 ,,	1·5 ,,
9	19	12	3	1	0	,,	,, (5·0 cm.)	163 ,,	1·5 ,,

Frequency distribution of multiple tracks.

In the Table I is given the result of our measurements on the frequency distribution of multiple tracks found in fifteen different plates. The number of prongs contained in each star is designated as the multiplicity of the star.

TABLE II

Frequency of multiples						Place	Absorbing substance
2	3	4	5	6	7		
0	8	10	2	1	1	Sandakphu Plate I	Air
0	2	3	2	0	0	,, ,, II	,,
0	2	4	7	0	0	Pharijong ,, I	2½ ft. wood and mud
0	8	5	6	0	2	,, ,, II	,, ,, ,,
0	5	6	1	2	0	Darjeeling ,, I	Air
2	4	4	3	0	0	Sandakphu	Water (20 cm.)
0	4	4	1	0	0	,,	Paraffin (20 cm.)
0	3	2	3	0	0	Pharijong	,, (20 cm.)
0	1	6	5	1	0	Sandakphu	Lead (·5 cm.)
1	1	8	3	0	0	,,	,, (1·0 cm.)
1	12	9	8	0	0	,,	,, (1·5 cm.)
0	8	6	0	1	0	,,	,, (2·0 cm.)
1	3	2	3	1	0	,,	,, (3·0 cm.)
2	4	3	2	1	0	,,	,, (5·0 cm.)
0	3	1	1	0	0	Darjeeling Plate II	Air

Number of tracks in the multiples tabulated in Table I in which the m.g.s. $\leqq 2{\cdot}4\,\mu$.

It was noted that there are a number of multiples which contain one or more than one track with very small grain spacing. Consequently the tracks given in the above table have been classified into groups. One group containing multiples having one or more than one track with m.g.s. $\lesssim 2{\cdot}4\times10^{-4}$ cm. And the other containing finer tracks only, i.e. with m.g.s. $> 2{\cdot}4\times10^{-4}$ cm.

In the Table II is given the data for those multiples which have at least one close grained track.

It was observed that these stars consist of both types of tracks, dense as well as fine grained, i.e. ionization loss in one type is greater than in the other.

Supposing in a multiple of m tracks p are found with the m.g.s. $\sim 2{\cdot}4\times10^{-4}$ cm., which are supposed to be due to protons of energies $\sim10^6$ eV, the meson multiplicity of the tracks therefore is taken as $n = m-p$. The total number of stars with a given meson multiplicity are then arranged in Table III according to increasing values of n; the energy ϵ' associated with the production of the multiple of 'm' tracks is $\epsilon' = n\cdot\mu c^2+p\cdot E_p+n\cdot E_\mu$, where $\mu c^2 = 10^8$ eV is the meson mass-energy E_p = kinetic energy of proton, $\sim10^6$ eV. E_μ = kinetic energy of meson $\sim10^6$ eV. The emulsion records proton and meson tracks of energy 10^6-10^7 eV only. Both E_p and E_μ are considered small compared to μc^2 and are ignored so that finally $\epsilon' = n\mu c^2$.

TABLE III

Frequency of multiple tracks						Place	Absorber.
2	3	4	5	6	7		
16+8 (24)	12+7 (19)	8+1 (9)	2+1 (3)	1+1 (2)	0	Sandakphu Plate I	Air
4+3 (7)	12+2 (14)	7+1 (8)	2+0 (2)	0	0	,, ,, II	,,
0+2 (2)	7+6 (13)	3+5 (8)	4+0 (4)	1+0 (1)	0	Pharijong I	2½ ft. mud and wood
3+6 (9)	10+6 (16)	8+3 (11)	3+2 (5)	0	0	,, ,, II	,, ,, ,,
5+9 (14)	13+3 (16)	4+1 (5)	2+2 (4)	0	0	Darjeeling ,, I	Air
2+3 (5)	4+1 (5)	4+1 (5)	0	0	0	,, ,, II	,,
6+5 (11)	4+3 (7)	1+2 (3)	1+0 (1)	0	0	Sandakphu	Water (20 cm.)
4+5 (9)	6+4 (10)	5+0 (5)	1+0 (1)	0	0	,,	Paraffin (20 cm.)
2+2 (4)	1+3 (4)	4+2 (6)	2+0 (2)	0	0	Pharijong	,, ,,
5+4 (9)	14+7 (21)	12+2 (14)	2+1 (3)	1+0 (1)	0	Sandakphu	Lead (·5 cm.)
7+4 (11)	21+5 (26)	11+4 (15)	5+0 (5)	1+0 (1)	0	,,	,, (1·0 cm.)
13+10 (23)	21+7 (28)	13+3 (16)	7+0 (7)	1+0 (1)	0	,,	,, (1·5 cm.)
9+7 (16)	13+4 (17)	12+0 (12)	5+0 (5)	1+0 (1)	0	,,	,, (2·0 cm.)
14+1 (15)	15+3 (18)	10+2 (12)	1+1 (2)	0	0	,,	,, (3·0 cm.)
7+4 (11)	15+3 (18)	9+2 (11)	1+1 (2)	0	0	,,	,, (5·0 cm.)

The figures given in brackets in different rows give the corrected frequency distribution of meson multiples occurring in the different plates.

The lower figures in brackets in each row represent the total number of multiples having tracks with m.g.s. $> 2{\cdot}4 \times 10^{-4}$ cm. The times of exposure of different plates and the areas examined are not equal. In order to find out what fraction of the primary radiation has been absorbed in traversing water (20 cm.), paraffin (20 cm.) and lead (5 cm.) we reduce them to equal time and area.

In Table IV we have given these results.

TABLE IV

Frequency of multiple tracks						Place	Absorber	Time of exposure	Area
2	3	4	5	6	7				
13·3	10·5	5·0	1·7	1·1	0	Sandakphu Plate I	Air	100 days	1 cm.²
5·5	3·5	1·5	0·5	0	0	,,	Water (20 cm.)	100 ,,	1 ,,
4·4	8·8	5·0	1·2	0	0	,, ,, II	Air	100 ,,	1 ,,
2·8	3·1	1·6	0·3	0	0	,,	Paraffin (20 cm.)	100 ,,	1 ,,
4·6	7·5	4·6	0·8	0	0	,,	Lead (5 cm.)	100 ,,	1 ,,
9·3	10·6	3·3	2·6	0	0	Darjeeling Plate I	Air	100 ,,	1 ,,
2·8	2·8	2·8	0	0	0	,, ,, II	,,	100 ,,	1 ,,
4·5	8	5·5	2·5	0	0	Pharijong ,, II	,,	100 ,,	1 ,,
2	2	3	1·5	0	0	Pharijong	Paraffin (20 cm.)	100 ,,	1 ,,

Frequency distribution of meson multiples in different plates per cm.² area per 100 days.

Sandakphu air Plate I, Darjeeling air Plate I, and Sandakphu water plate are taken from the same batch. And Sandakphu air Plate II, Pharijong plates and those exposed under paraffin are from another batch.

Discussion.—The most striking fact which has come to our notice in the Table III is that when the tracks are grouped according to their multiplicity in majority of plates the frequency of multiples with three prongs is found to be maximum. We have indicated in §3 that the total energy ϵ_n of a star containing n mesons will be approximately equal to $n \cdot \mu c^2$, where μc^2 is the mass-energy of the mesons produced, neglecting kinetic energies of the secondary particles. Hence the present experiment reveals the fact that the maximum production of multiple mesons occurs at energy $\sim 3\mu c^2$.

This experimental result appears to be in qualitative agreement with the recently published theory by Heitler, Hamilton and Peng (1943). We have given a preliminary account of Heitler's theory in §2, so far as the mechanics of single and multiple meson productions due to scattering of fast protons by nuclear particles are concerned. The theory has been applied to deduce a theoretical expression $\psi(\epsilon, x)$ connecting the number of mesons of energy ϵ and $d\epsilon$ found at different depth x from the top of the atmosphere; x is expressed in terms of a unit thickness of matter in which a fast charged particle loses by ionization one unit of energy $\mu c^2 = 1$. The meson field of a proton consists chiefly of charged transverse and pseudoscalar components whose coupling constants with a nuclear particles are denoted by $f^2 = f'^2$ and their numerical values taken from the theory of nuclear forces due to Möller and Rosenfeld (1941) are $f^2 = f'^2 = 0{\cdot}13$. The decay constants of the corresponding mesons are taken to be 10^{-8} sec., 10^{-6} sec., respectively, so that in the lower reaches of the atmosphere mostly pseudoscalar mesons are to be found.

The primary cosmic ray particles incident on the top of the atmosphere and responsible for the multiplicity of cosmic ray effects observed in the atmosphere are taken to be high

energy protons with an integral energy distribution $F(E)dE = A.dE/(E/43 \log 0{\cdot}3E)$; $\psi(\epsilon, x)$ the distribution for meson spectrum satisfies the diffusion equation

$$\frac{\partial \psi}{\partial x} = \frac{\partial \psi}{\partial \epsilon} - \frac{b}{\epsilon x}\psi + S$$

which is similar to that given by Euler and Heisenberg excepting for the source function S. From a solution of the diffusion equation, we can obtain theoretically the function $\psi(\epsilon, x)$ which gives the meson energy spectrum ϵ at a depth x. By graphical solution two curves have been drawn for $x = 22$ and $x = 15$ from the top of the atmosphere; the former is compared with the results of Blackett for small and medium energy particles. The curve has a maximum for $\epsilon = 10$ in agreement with Blackett's results, then it bends down and at low energy $\epsilon \sim 3$, there is again a rise of the curve which again bends towards zero. The position of the second maximum at $\epsilon \sim 3$ in the energy spectrum coincides with our experimental result. The reason for the occurrence of the secondary maximum at $n = 3$ may be due to the following. In §2, we have seen that mesons are emitted as a result of proton-proton collision. It has been found that in treating these collision processes it is necessary to consider the effect of the reaction of the emitted meson on the motion of the nucleon. For mesons with energy $\epsilon > \frac{1}{f}$ but $< M$, radiation damping correction is found necessary; the cross-section is found to vary inversely as the square of the energy, i.e. $\frac{1}{\epsilon^2}$. When primary proton energy $E > M$, then for mesons with energy $\epsilon \gtrsim \frac{1}{f}$ the cross-section for production can be calculated by neglecting the damping effect and it is found to vary with the square of the energy, i.e. $\sim\epsilon^2$. Thus the function $\phi(\epsilon')d\epsilon'$ representing the cross-section for production of mesons must have a maximum somewhere in the region approximately at $\epsilon \sim 3\mu c^2$. After that it falls rapidly to zero for $\epsilon \to 0$.

The most reliable measurements for the low energy meson spectrum appear to be those due to D. J. Hughes (1940). His energy distribution curve shows a maximum near $\epsilon' \sim 8 \times 10^8$ eV; the number of penetrating particles of lower energies diminish rapidly. This is not in agreement with the predictions of the theoretical $\psi(\epsilon, x)$ curve. If we now turn to the results tabulated in Table III we find the frequency distribution curve for multiple meson production has a maximum between $n = 2$, $n = 3$. According to theory the energy spectrum for the occurrence of n mesons is given approximately by the function $\psi(\epsilon, x)\frac{\gamma_n}{\gamma_1}$, where γ_n is the cross-section for the production of n mesons by scattering and γ_1 the cross-section for single meson scattering. Thus the occurrence of a maximum at $n \sim 3$ may be taken as a qualitative verification of the theoretical curve $\psi(\epsilon, x)$ given by Heitler *et al.*

Another interesting relation to which attention may be drawn is (i) in none of the plates any multiple with $n > 7$ has been observed with the exception of two instances of emission of a large number of particles, one containing 13 and the other not less than 30 which may be due to some other process; (ii) in single meson spectra obtained from Wilson Chamber photographs there is a maximum for $\epsilon \sim 8 \times 10^8$ eV after which in the region of lower energies the meson spectrum falls away rapidly. A possible correlation may exist between these two results which may be explained by either of the following two interpretations: (*a*) single mesons in air of energies between $3\mu c^2$ and $8\mu c^2$ have large

cross-section for multiple meson production by scattering in a nuclear field; (*b*) protons of energy between the above two limits have much larger scattering cross-section for multiple meson production compared to single meson production. The one will account for paucity of single mesons in air with the given energy range, and the other for the larger production of such meson multiples in photographic emulsion.

Lead Plates.—Heitler *et al.* (1939) in an experiment with photographic plates, kept under different thicknesses of lead, observed a maximum for single tracks at lead thickness 1·2 cm. In our investigations with lead absorbers we have examined all multiple tracks produced in the plates under different thicknesses of lead, viz. from 0·5 cm.→5·0 cm. It is observed there that frequency of multiple tracks with multiplicity up to 6 has a maximum under 1·5 cm. Pb. This maximum coincides with the first maximum of Rossi's curve. A plausible interpretation of this result is that photons with energy $\sim 10^9$ eV after traversing lead plates can produce soft mesons along with cascade showers.

The possibility of photons as one of the sources of meson production has been suggested by many investigations. In course of an investigation Schein, Jesse, Wollan (1941) reported that in 2 cm. lead block photon component of cosmic rays can create mesons. The production begins noticeably at about 6 km.; we have observed meson production at much lower altitude than this. They found the cross-section for production of meson (3·5 km.) of the order of 10^{-27} cm.^{2} Energy of the created mesons they recorded as always higher than $1{\cdot}2\times 10^8$ eV. Many other investigators have found evidences of occurrences of mesons in big cascade showers. They are seen to be most frequent at the maximum position of cascade production.

Hamilton and Peng (1944) have calculated cross-section of production of pseudo-scalar mesons by energetic photons as $\sqrt{2}\,.\,\pi^2 ef/\epsilon^2$. The cross-section is very small in comparison with soft pair production.

Tabin (1944) has reported the results of an experiment on the production of mesons at altitudes of 10,000 ft. and 14,200 ft., under different thicknesses of lead, iron and paraffin. His conclusion is that at these altitudes photons are one of the chief agents for the production of mesons. He found the cross-section for creation of meson in lead is of the order of $\sim 10^{-24}$ cm.^{2} and in iron and paraffin $\sim 10^{-25}$ cm.^{2}, much higher than according to Heitler's views. According to Tabin's results mesons are generated by collision of photons with the atomic nucleus of the producing material. There is also a clear indication that at 1·2 cm. lead the creation of soft mesons becomes maximum. This is in good agreement with our results, which show a similar maximum for mesons with kinetic energy $\sim 10^6 \rightarrow 10^7$ eV.

It is observed in Table III that even in plates under various thickness of lead, the frequency of meson production reaches a maximum at the energy region $\sim 3\mu c^2$.

It is known that a light quantum through a collision with a nucleon produces a meson in a process like $h\nu + \mathrm{P} \rightarrow \mathrm{N} + \mu^+$. It is found that if radiation damping is neglected, the cross-section would increase with square of the energy of the incident photon, but the experimental results speak otherwise.

Hamilton and Peng (1944) have calculated the cross-section for production of a meson of energy ϵ by collision of a photon of the same energy with a nucleon. They found that the cross-section for production of a meson of any polarization can be obtained from ordinary theory, neglecting damping; it increases with ϵ^2 if energy $\epsilon \gg 1$ but $e^2 f^2 \epsilon^4 \ll 1$ (e is the elementary electric charge); but for high energies where damping effect is prominent, i.e. when $e^2 f^2 \epsilon^4 > 1$, the cross-section is proportional to inverse of ϵ^2

for transverse and pseudoscalar mesons. So we expect the cross-section to be maximum somewhere between the two limits

$$\epsilon \lesssim \frac{1}{\sqrt{ef}} \text{ and } \epsilon > \frac{1}{\sqrt{ef}}.$$

This theory is based on two assumptions: (i) ϵ will always be $< 10^9$ eV, (ii) mesons will always be charged. Actually in our experiment we find a maximum position at $\epsilon \sim 3 \times 10^8$ eV.

Absorption of Primary Radiation

§4. Further information regarding the nature of primary radiation has been obtained from absorption measurements in different materials. Considering the data given in Table IV, we are led to the belief that it is not only the photon component but some penetrating component, either (i) mesons or (ii) nucleons (ionizing or non-ionizing), is also active in producing secondary multiple tracks inside the photographic emulsion.

From the data given in Table IV an estimate can be made of the absorption of primary energy in absorbers such as 20 cm. paraffin, 20 cm. water; 5 cm. lead and the mass of air between the two altitudes Sandakphu and Darjeeling. In order to decide which of the penetrating components is chiefly responsible for the production of secondary particles in photographic plates the following procedure has been adopted.

Consider a thickness t of an absorbing substance. It is assumed that the cross-section of the cosmic ray component, responsible for the production of multiple tracks in the photographic emulsion, is independent of the energy E_0 of the former and is given by $E = E_0 e^{-\alpha t}$, where E_0 is the energy incident per cm.^{2} of the surface in 100 days; the energy absorbed in the thickness t of the absorber is

$$E_t = E_0 - E = E_0 (1 - e^{-\alpha t}) \quad .. \quad .. \quad .. \quad .. \quad (5)$$

On the top and under this absorber photographic emulsion plates of thickness $\delta t \ll t$ are placed.

If N_1 is the total number of tracks, all supposed to be due to mesons, produced per cm.^{2} in 100 days in the upper plate, and N_2 the total number in the lower plate, and α_0 is the coefficient of absorption of the effective radiation in the emulsion and α that in the absorber. Then

$$N_1 \times 10^8 = \alpha_0 E_0 . \delta t \quad .. \quad .. \quad .. \quad .. \quad .. \quad (6)$$

$$N_2 \times 10^8 = \alpha_0 E . \delta t \quad .. \quad .. \quad .. \quad .. \quad .. \quad (7)$$

Since the photographic emulsion is rich in hydrogen and carbon we can take $\alpha_0 = \alpha$ for paraffin approximately. From these equations we can obtain values of

$$\alpha = \frac{1}{t} \log \frac{N_1}{N_2} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (8)$$

$$E_0 = \frac{(N_1 - N_2) \times 10^8}{\alpha . \delta t (1 - e^{-\alpha t})} \quad .. \quad .. \quad .. \quad .. \quad (9)$$

Further $\frac{E_t}{t} = E'$ is the energy absorbed per unit thickness per unit area of the absorber per 100 days. The average energy per incident particle is $\frac{E_0}{N}$, where N is the number of primary particles incident per unit area per 100 days and $\frac{E'}{N}$ the average energy absorbed per cm. per each primary particle of average energy $\frac{E_0}{N}$. In order to find the number N

of penetrating particles falling per unit time per unit area at our place of observation, we have taken an energy distribution formula for the primary particle, similar to that for the meson spectra, as given by Christy and Kusaka (1939).

According to these authors the number of penetrating particles of energy between ϵ and $d\epsilon$ incident on unit area per solid angle $d\Omega$ per unit time at a depth T from the top of the atmosphere is given by

$$N(\epsilon)\, d\epsilon\, d\Omega = \frac{\cdot 02\,(\epsilon_c)^{\gamma} \cdot d\epsilon \cdot d\Omega}{(\epsilon + aT)^{\gamma+1}} \qquad \ldots \quad \ldots \quad \ldots \quad (10)$$

where ϵ_c is the critical cut off energy corresponding to $\epsilon_c \sim 3\mu c^2$ and is equal to $(1{\cdot}89+0{\cdot}3)10^9$ eV. We further take $\gamma = 2$.

Integrating this equation between the limits infinity and 3×10^8 eV, we get the total number of mesons incident per cm.^{2} per 100 days with energy $>3\times10^8$eV, at the depth 6·5 m. of water from the top as $1{\cdot}68\times10^5$.

We shall now show that the primary beam cannot consist of mesons.

(i) Dividing E_0 by the above number we get the average energy per incident meson to be $1{\cdot}18\times10^8$ eV, which is below the cut off energy of the meson spectrum.

(ii) As will be seen from calculations given in Table V the absorption of primary radiation is in the following order of increasing magnitude lead < air < paraffin < water, i.e. absorption is inversely as the average atomic weight of the absorber, being maximum in hydrogenous substances. This gives a strong support to the assumption that the primary effective radiation is nucleon (proton/neutron).

(iii) Another reason for which mesons cannot be considered as primary particles has been discussed by Heitler (1939). In an investigation with photographic plates he found that frequency of tracks in photographic plates increases as the soft component of cosmic rays between the altitude of Bristol and Jungfrau Joch, while it is known that intensity of meson component increases only 1·7 times from sea-level to the elevation of 6 m. water.

We shall now make the assumption that the primary radiation is proton (neutron), and on this basis calculate the energy loss suffered per each primary particle when traversing unit thickness of an absorber. There is no direct method of estimating the energy distribution in the proton spectrum at different altitudes. We assume that the distribution follows an exponential law like that for meson spectrum, and further we take the proton number to be a fraction of the meson number.

Johnson (1940) and others conclude from their Wilson Chamber photographs of slow protons, that the number of protons is about 2% of the total penetrating component of cosmic radiation; this estimate is supported by Shutt (1942) from his investigations on the anomalous scattering of protons in lead plates placed inside a Wilson Chamber. On the other hand, Wilson (1939) from his absorption measurements of the penetrating component in different dense elements in Wilson Chamber finds that in a number of particles of energy $>7\times10^8$ eV, the energy loss is several times larger than that due to ionization only. It is probable that these particles are protons. He calculates that they represent 7% of the incident penetrating particles. Seren (1942) has estimated the proton component at sea-level to be about 5% of the meson component. As a mean of the different estimates we assume that 5% of the primary penetrating components are protons.

From which it follows that the number of protons incident at 6·5 m. water depth per cm. per 100 days is $8{\cdot}5\times10^{8}$. The average energy per incident particle then comes out to be of the order of 10^{9} eV, which is quite reasonable.

TABLE V

No.	Absorbing substance	No. of tracks per cm.^{2} per 100 days	Coefficient of absorption α	Average energy per incident particle E_0/N	Energy loss per cm. of incident particle E'/N	Ionization loss per cm. of proton of energy 10^9 eV	Ratio between V & VI	Depth from top of atmosphere in metres of H_2O
1	Air ..	62 (Plate II)	..	$2{\cdot}17\times10^{9}$ eV	..	..	..	6·5
2	Paraffin (20 cm.)	23	·049 (*a*)	„	$6{\cdot}9\times10^{7}$ eV	$2{\cdot}4\times10^{6}$ eV	29	6·5
3	Air (8×10^{4} cm. at N.T.P.)	24	$1{\cdot}24\times10^{-5}$ (*b*)	„	$1{\cdot}8\times10^{6}$ eV	$2{\cdot}4\times10^{8}$ eV	7·5	7·9
4	Lead (5 cm.) ..	54	·032(*c*)	„	$6{\cdot}5\times10^{7}$ eV	$1{\cdot}4\times10^{7}$ eV	4·6	6·5
5	Air ..	93 (Plate I)	..	$2{\cdot}9\times10^{9}$ eV	..	..	..	6·5
6	Water (20 cm.)	30	·055(*d*)	„	$9{\cdot}7\times10^{7}$ eV	$2{\cdot}3\times10^{6}$ eV	41	6·5
7	2½ ft. wood and mud.	67·5	..	$2{\cdot}4\times10^{9}$ eV	..	..	..	6·1
8	Paraffin and 2½ ft. wood and mud.	29·5	·041(*e*)	„	$6\ 7\times10^{7}$ eV	$2{\cdot}4\times10^{6}$ eV	28	6·1

Energy loss calculations; column III by formula (8, §4); column IV by formulae (9 and 11, §4) and column V by formula (5, etc., §4).

(*a*) Paraffin, Sandakphu.
(*b*) Air mass between Sandakphu and Darjeeling.
(*c*) Pb, Sandakphu.
(*d*) Water, Sandakphu.
(*e*) Paraffin, Pharijong.

In Table V, we have given the results of our calculations of the energy loss per cm. length in different absorbers of primary particles of average energy of the order of 10^9 eV. In the column VII we have expressed the calculated energy loss per cm. length of the incident proton in different absorbers in terms of the ionization loss suffered by such protons. This ratio is of interest in view of a recent rough estimation given by Heitler *et al.* (1943) of the energy loss suffered by protons of energy of order of proton mass, i.e. 10^9 eV. It is supposed according to this theory, that each nucleon of energy E carries with it a meson field a part of which ϵ is radiated away as free mesons when a fast proton of energy E collides with a stationary one. The calculation is valid for proton energy $E \gg M \sim 10^9$ eV, where M is the proton mass-energy. At this stage though meson production is negligible compared to its rate at higher energies the energy loss still would be ten times greater than that due to ionization only.

In addition to the energy loss of the primary proton due to ionization and meson production, a part of the energy may be transferred as mechanical momentum to the stationary nucleon. This transference will be the largest when the stationary nucleon is free. If on the other hand it forms part of a heavy nucleus of mass M, the average momenta transference will be as 1/M.

We are now in a position to discuss the results given in Table V. The proportional energy loss of the primary particle in different absorbers given in the column VII are as follows:—

Lead	Air	Paraffin	Water
4·6	7·5	28 ; 29	41

They are all of the expected order of magnitude, viz. 10; further the energy losses are inversely as the atomic weights of the substances. The abnormally low value for lead may be due to the fact that we have measured the absorption in 5 cm. thickness of lead and some of the photons produced in this transition layer may not have been completely absorbed in it and thus become effective in producing multiple tracks in the photographic emulsion. The absorption in paraffin measured at two different places, Sandakphu (12,000 ft.) and Pharijong (14,500 ft.), appear to agree well. It is not possible to explain why absorption in water is so much greater than that in paraffin. We kept a photographic plate at Sandakphu under 18 cm. of carbon with a view to separating the absorption due to hydrogen from that due to carbon in paraffin, but the plates being about three years old had lost their sensitiveness. We hope to repeat these experiments when fresh batches of Ilford New Halftone plates become available. The present agreement obtained between the prediction of Heitler's theory and our experimental results depends chiefly on our assumption that at the altitude of our measurements 5% of the penetrating particles are protons. On the other hand, the experimental results that the absorption varies inversely as the atomic weight is a good indication that the effective component of the cosmic rays for the production of multiples in photographic plates is protonic in character.

Whether these low energy protons, effective in producing multiple tracks in photographic emulsion, form a part of the primary beam incident on the top of the atmosphere or are secondaries produced in the lower altitudes by some photonuclear process cannot be decided definitely. The possibility of such a process in the atmosphere is suggested by Korf. Heitler's observation that the intensity of tracks in photographic plate increases with altitude as soft component also supports the view that these may be generated by the photon component and are not a part of the primary protons incident at the top of the atmosphere.

Cross-section for Meson production.—We have calculated the cross-section for the production of mesons in photographic emulsion by protons in the following manner:—

Let y be number of events producing multiple tracks in the photographic emulsion, the multiplicity n varying from $n = 2$ to 7. Then $y = 2\pi\sigma\ N\alpha\,.\,At$, where σ is number of nuclei per c.c. of the emulsion $= 1{\cdot}4\times10^{23}$; N = number of incident protons per cm.^{2}; A = average atomic weight of the emulsion = 12; t = thickness of the emulsion = 70×10^{-4} cm.; α = cross-section for production of mesons per nucleon inside the emulsion.

$$\alpha = \frac{y}{2\pi\sigma NAt} \qquad (11)$$

the value of α is found equal to $3{\cdot}2\times10^{-26}$ cm.^{2} per nucleon.

Heitler and Peng (1942) have calculated the integral cross-section for production of mesons of energy $\epsilon \gtrsim M$ but always $> \frac{1}{f}$ by proton-proton collision, if energy of fast proton be $E \gg M$, as $2{\cdot}9\times10^{-25}$ cm.^{2}, which is very large. But they have predicted that in the case of $E < M$ the cross-section can be calculated by neglecting damping effect and

will be much lower than this value. The production cross-section will decrease rapidly with the decrease of energy E. Our experimentally found cross-section is one-tenth of the theoretically calculated one. It appears to be a reasonable value; since the energy loss in our region ($E \sim M$) is about one-tenth of that of protons ($E \gg M$), for which the theoretical calculation is made.

Nordheim and Nordheim considered the cross-section for production of mesons by proton-proton collision in non-relativistic region without taking any account of radiation damping and found it to be of the order of 10^{-28} cm.^{2}, which is much smaller than that found by us. On the other hand Shutt (1942) in a Wilson Chamber experiment on scattering of penetrating particles with energy range $10^8 \rightarrow 10^9$ eV in lead noted that a certain fraction of the particles suffered larger scattering in 5 cm. Pb than what can be expected on the basis of the theory of Coulombian scattering. Attributing this anomaly in scattering to non-electric nuclear forces, the average cross-section per nucleon for mesons has been found to be of the order of $6{\cdot}5 \times 10^{-28}$ cm.^{2} It agrees well with the result obtained by Code (1941). Further it was noted that the number of tracks scattered in this anomalous way amounts to about 2% of the whole, which is of the same order as the estimate of the proton component made by Johnson *et al.* Further Wilson (1939) has shown that the scattering of about 7% of the penetrating component cannot be explained as due to multiple electric scattering. From these considerations Shutt has drawn the conclusion that it is possible that a large fraction, or even the whole of the anomalous large-angle scattering observed, is associated with the proton component. Further, if the cross-section for proton-proton or proton-neutron scattering at cosmic ray energies is taken to be 100 times larger than that calculated for mesons, large angle scattering can be explained on the assumption that cross-section of protons scattering per nucleon is of the order of 10^{-26} cm.^{2} Our value also comes to the same order of magnitude.

Summary and Conclusions

§5. This paper gives an account of investigations undertaken with Ilford Halftone plates, which were exposed to cosmic rays at different places, Darjeeling (7,000 ft.), Sandakphu (12,000 ft.) and Pharijong (14,500 ft.), under different absorbers like air, water, paraffin and lead. The characteristics of the heavy ionization tracks found in them are discussed, and reasons are given for assigning the tracks: (i) along which the mean grain spacing (m.g.s.)$\leqq 2{\cdot}4\ \mu$ to protons and α-particles, and (ii) along which the m.g.s. $> 2{\cdot}4\ \mu$ mainly to mesons.

The frequency distribution of meson multiple tracks with $n = 2$–7 has been determined, and it is shown that this distribution has a maximum for $n \sim 3$, which corresponds to the energy of the primary radiation $\sim 3 \times 10^8$ eV. Attention is drawn to the existence of a similar maximum in the region of 3×10^8 eV in the energy distribution curve of the single meson spectra theoretically deduced by Hamilton, Heitler and Peng (H.H.P.) for different depths from the top of the atmosphere. According to this theory the primary cosmic ray particle responsible for meson (including meson shower) production is proton.

The absorption of the radiation, responsible for multiple meson production in photographic plates, in absorbers like air, water, paraffin and lead has been measured. The average energy loss in a given medium for each primary particle of energy $\sim 10^9$ eV has been expressed as a multiple of the ionization loss suffered by protons of energy 10^9 eV in the same medium, and the following results have been obtained:—

Lead	Air	Paraffin	Water
4·6	7·5	28; 29	41

The result obtained is in qualitative agreement with the estimate of H.H.P. for the energy loss of protons of energy 10^9 eV, which was found to be ten times the ionization loss. The energy loss is also inversely proportional to the nuclear mass of the absorber, being maximum in hydrogen containing substances, showing that protons (neutrons) are mainly responsible for such multiple production.

The average cross-section for the production of meson showers by proton particles of mean energy $\sim 10^9$ eV has been found to be $3{\cdot}2 \times 10^{-26}$ cm.^{2}, per nucleon, which is one-tenth of the value calculated by H.H.P. for the average cross-section ($2{\cdot}9 \times 10^{-25}$ cm.^{2}) for protons of the energy $E > 10^9$ eV. Reason for the discrepancy is discussed.

The effect of interposing lead sheets of thicknesses 0·5 cm. to 5·0 cm. on multiple production has been studied. It is found that (i) the total number of tracks produced per unit area has a maximum for 1·5 cm. Pb, and (ii) for each thickness of Pb, the meson multiple frequency curve has a maximum at $n = 3$; (i) indicates that photons can also produce multiple mesons. The implication of (ii) is discussed in light of Heitler and Peng's theory of meson production by light quanta.

The author desires to express her sincere thanks to Dr. D. M. Bose under whose guidance the work was carried out.

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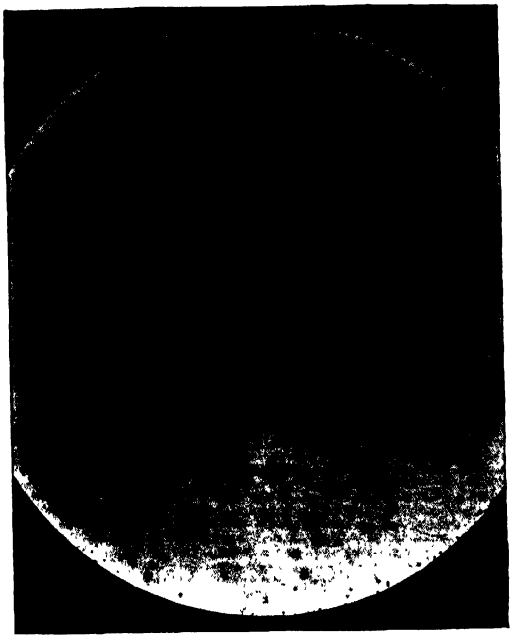
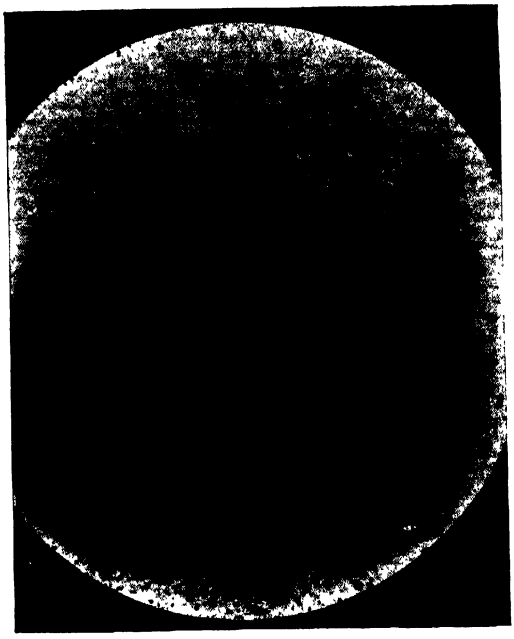


I
Sandakphu : single curved track.



II
Sandakphu : pair track.

III
Sandakphu : 5 star multiple one heavy particle m.g.s. $< 2{\cdot}4\mu$.

IV
Darjeeling : 12 star multiple.

Examples of ionization tracks obtained on Ilford New Halftone plate exposed to cosmic rays at Sandakphu (alt. 12,000 ft.) and Darjeeling (alt. 7,000 ft.).

VII. ON THE DETECTION OF SPONTANEOUS FISSION OF URANIUM NUCLEUS

By S. D. CHATTERJEE

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INTRODUCTORY

The phenomenon of induced fission of heavy nuclei like Uranium and Thorium, by neutron bombardment, are now quite well-known. The idea of such a process was first put forward by Noddack (1934), rather as speculation, in order to criticize Fermi's conclusions about transuranic elements. It took a definite shape when Hahn and Strassmann (1939), as a result of elaborate work on the chemical properties of the so-called 'transuranic' elements, were forced to suggest that most probably the Uranium nucleus broke up into two parts of comparable sizes, a Barium nucleus ($Z = 56$) and a Krypton nucleus ($Z = 36$). These unexpected and startling results, which were offered with much reserve, led others to perform experiments of diverse characters, which were soon to give abundant evidence for the correctness of Hahn and Strassmann's conclusions. It was soon realized by many experimenters that such a splitting of heavy Uranium or Thorium nucleus into lighter elements would involve the release of an enormous amount of energy, since the masses of practically all possible pairs of lighter elements were less than that of the original one.

Frisch (1939) detected directly the heavily-ionizing recoil fission products, using a Uranium-lined ionization chamber connected to a linear amplifier. The output of the amplifier was connected to a thyratron, which was biased so as to count only pulses corresponding to more than 5×10^5 ion pairs. No such bursts of ionization were observed until a (Ra-Be) source of about 300 mc. was placed near the ionization chamber, when about 15 particles per minute were recorded. From a measurement of the energy loss, corresponding to the maximum size of the pulse, he estimated that the particles concerned must have an atomic weight greater than 70. Thus, conclusive physical evidence of the breaking up of the Uranium nucleus into two parts of comparable size was obtained. This type of experimental evidence was also obtained soon after, by Roberts, Meyer and Hafstad (1939) and Green and Alvarez (1939). Booth, Dunning and Slack (1939) also investigated the energy distribution of the fragments resulting from Uranium fission under well-defined conditions.

The first theoretical interpretation of these observations was given by Meitner and Frisch (1939). In accordance with Bohr's (1936) ideas about the behaviour of heavy nuclei, they suggested an essentially classical picture of the new disintegration process. On account of their close packing and strong energy exchange, the particles in a heavy nucleus would be expected to move in a collective way which has some resemblance to the movement of a liquid drop. If the movement is made sufficiently violent by adding energy, such a drop may divide itself into two smaller drops. They also introduced the concept of surface tension of nuclear matter in the discussion of energies involved in the deformation of nuclei and showed that the surface tension of nuclei, decreasing with increasing nuclear

charge, might become zero for atomic numbers of the order of 100. In fact, the nucleus, like a liquid drop, is subject to the following forces:—

(i) A short range non-electrical force, only acting between the neighbouring particles: this is proportional to the total number A of the particles in the nucleus: it can be represented by $-\alpha A$;

(ii) A term which represents the decrease of binding energy when the number of protons and neutrons become different and be represented by $\beta(N-Z)^2/A$. This holds truly for $(N-Z) \ll A$;

(iii) A force analogous to surface tension, since particles at the surface interact on the average only with half as many other particles as do particles in the interior, and represented by $\gamma A^{\frac{2}{3}}$; and

(iv) A repulsive coulomb force proportional to the square of Z, viz.

$$\frac{3}{5}\left(\frac{e^2}{r_0}\right)\frac{Z^2}{A^{\frac{1}{3}}};$$

where $r_0 A^{\frac{1}{3}}$ is the nuclear radius.

Thus the energy of formation of a nucleus containing N neutrons and Z protons is given by the empirical formula

$$\alpha A - \frac{\beta(N-Z)^2}{A} - \gamma A^{\frac{2}{3}} - \frac{3}{5}\left(\frac{e}{r_0}\right)\frac{Z^2}{A^{\frac{1}{3}}} \cdots\cdots (k),$$

as given by Bethe.

The actual nucleus will be stable so long as the sum of the electrostatic and surface tension energy has a minimum for the spherical configuration of the nucleus. With increasing size and charge of the nucleus, this minimum would flatten and would be expected to disappear for some critical value of Z. Nuclei of greater Z would break apart. Bohr and Wheeler (1939) have considered the stability of the nucleus (A, Z) against small arbitrary deformations of the simplest type. They find that with increasing value of $\frac{Z^2}{A}$ we come to a limiting value,

$$\left(\frac{Z^2}{A}\right)_{\text{limiting}} = 10\left(\frac{4\pi}{3}\right)\frac{r_0^2 O}{e^2} \cdots\cdots (l),$$

beyond which the nucleus is no longer stable with respect to deformations of the simplest type. The quantity $4\pi r_0^2 O \sim 14$ eMV is to be identified with constant γ in equation (l); O then represents the surface tension of the liquid drop nucleus. They find that this ratio is greater (by 17%) than the value of $\frac{Z^2}{A}$ for U^{238}. Such nuclei are therefore near the limit of stability and it is possible to calculate the potential necessary to deform the nucleus sufficiently to produce division. The value of this deformation potential has been calculated for $_{90}$Th, $_{91}$Pa and $_{92}$U and they are found to vary between 6·9 eMV for $_{90}Th^{232}$ to 5·0 eMV for $_{92}U^{235}$. When a neutron is captured by such nucleus, an amount of energy of the order of 8·5 eMV (on an average) is released; the compound nucleus thus formed is raised to an excited state with this excess of energy (8·5 eMV) over the ground level and if the latter is greater than the energy of fission E_f, fission will occur. The detailed comparison of the calculated probabilities of $_{92}$U, $_{91}$Pa and $_{90}$Th nuclei lead to good agreement with experimental results. The results obtained so far are based purely upon classical mechanical consideration which is in agreement with the fact that zero-

point energy of the nucleus is found to be about $\frac{1}{15}$th of the energy of fission E_f. The statistical distribution in size of the fragments of fission depends on the complicated dynamics of the dividing nucleus. The theory is not developed enough to give this distribution, but does indicate that there is a wide range of possible fragments even for energies slightly greater than the critical energy.

Turning to the spontaneous fission of the Uranium nucleus, it is reasonable to suppose that the relevant theory must be analogous to the theory of α-activity of heavy nuclei, first developed by Condon and Gurney (1929) and Gamow (1929).

The theory of α-activity is the result of an application of quantum mechanics to the problem of motion of helium nucleus under the forces which the residual nucleus exerts upon it. In leaving the nucleus the α-particle, after overcoming the nuclear attraction, is subjected to the repulsion of the electrostatic (coulomb) field of the nucleus. The joint effect of these forces is that the particle must cross a 'potential' barrier and the quantum mechanical treatment showed that a particle has a finite probability of doing so even if the energy is too small to surmount the barrier. The probability for this 'tunnel effect' increases rapidly with increasing particle energy, in quantitative agreement with the experimental facts which are summarized in the Geiger-Nuttall relation $\log \lambda = A + B \log E$, which connects the decay constant λ of the substance with the energy E of the α-particles emitted. Bohr and Wheeler (1939) have pointed out that the distortion which leads to fission, although associated with a greater effective mass and lower quantum frequency, and thus nearly deserving classical considerations, will still be characterized by certain specific quantum properties. From the point of view of nuclear stability, the possibility of quantum-mechanical 'tunnel effects' will be quite important, which will make it possible for a nucleus to divide even in its ground state by passage through a portion of configuration space where classically the kinetic energy is negative. An accurate estimate for the stability of a heavy nucleus against fission in its ground state will, of course, involve a very complicated mathematical problem. In a natural extension of the above-mentioned theory of α-decay, they have, in principle, determined the probability per unit time of a spontaneous fission process λ_f, adopting certain simplifying assumptions which includes that the expression (V−E) in the Gamow function is of the order of the fission energy E_f, i.e. ~ 6 eMV. They find a mean lifetime against fission in the ground state $\frac{1}{\lambda_f}$ equal to ~ 10^{22} years.

Libby (1939) undertook experiments to detect unstable isotopes resulting from the natural fission of Uranium and Thorium nuclei. They concluded that the spontaneous fission, if any, must have a half-life of the order of at least 10^{14} years. The first definite evidence of the spontaneous fission of the Uranium nucleus was obtained by Petrzhak and Flerov (1940). They used an ionization chamber in the form of a many-plated condenser, whose plates were covered with a layer of U_3O_8. They connected the insulated plates of this ionization chamber with a high-gain linear amplifier of extremely high resolution. They observed a small number of pulses per hour, which they ascribed to spontaneous fission of Uranium, because a series of control experiments seemed to exclude other possible explanations.

The present investigation was undertaken with the following purposes in view:—

(A) Detection and identification of the large impulses in an ionization chamber due to the spontaneous fission of the Uranium nucleus.

(B) Determination of its half-life period. These experiments are described below.

EXPERIMENTAL

A. *Detection of spontaneous fission pulses of U.*

For this purpose, an experimental arrangement somewhat similar to that described by Petrzhak and Flerov (1940) was adopted. It consisted of the following:—

(i) An ionization chamber in the form of a many-plated condenser ;
(ii) A high-gain proportional amplifier ;
(iii) A Cathode ray oscillograph ; and
(iv) A thyratron-operated telephone call-counter.

The ionization chamber was constructed in the form of a shallow condenser with 15 brass plates (7 cm. × 7 cm. each), the separation between alternate plates being about 3 mm. Only the top surfaces of these plates were coated with U_3O_8, so that every sq. cm. contained 10–20 mgm. of the oxide. The plates were supported by brass pillars, passing through amber buttons embedded in a thick ebonite disc. The ionization chamber was encased within a vacuum-tight glass envelope, filled with nitrogen at atmospheric pressure. A photograph of the chamber is shown in Fig. 1A. Alternate plates were connected to the H.T. and the grid of the first tube of the proportional amplifier. The H.T. on the chamber was only +450 volts. In order to avoid the recording of impulses due to the superposition of the individual α-particles, the time-constant of the first coupling stage was made extremely small. In fact, the coupling condenser between the first tube and the second was only 20 cms., while the total amplification factor of the proportional amplifier was about 10^6.

When a (Ra–Be) neutron source was brought near the ionization chamber, large fission pulses could be easily distinguished against a background of much smaller α-ray pulses, as observed on the screen of a cathode-ray tube. The output of the amplifier was connected to a gas-triode, whose grid was negatively biased to such an extent that only the large fission pulses were capable of tripping it. It was observed that a small number of impulses (~3 per hour) continued to be registered even when the neutron source was removed from the neighbourhood of the ionization chamber. The origin of these spontaneous impulses might be attributed to the following causes:—

(1) External oscillations in the amplifier.
(2) Superposition of the individual α-particles.
(3) Appearance of sudden gas amplification in the ionization chamber.
(4) Accidental discharge on the surface of the Uranium oxide.
(5) Induced fission of Uranium by the neutron component of the cosmic rays.
(6) Natural fission of Uranium.

Control experiments showed that none of these causes, except the last one, could satisfactorily explain the observed effect. Some of these experiments are briefly enumerated below:

(*a*) When the brass plates of the ionization chamber were not coated with U_3O_8, no pulses were registered during a run of several hours.

(*b*) When the amount of U_3O_8 was reduced (by reducing the thickness of the layers or the number of the condenser plates), the number of spontaneous impulses diminished. Similar diminution in the number of induced fission pulses was also observed when a (Ra–Be) source was kept near the chamber.

(*c*) When the negative grid bias of the gas-triode was so increased that the induced fission pulses were not recorded, the spontaneous impulses also ceased to be recorded.

(*d*) Photographical registration showed that the induced fission pulses and the spontaneous impulses were of approximately the same size, Figs. 1B and C.

(*e*) Strong γ-ray source, consisting of several spent radon tubes, had no influence on the number of spontaneous impulses per hour.

(*f*) No change in the average number of spontaneous impulses per hour was observed, when

(i) the ionization chamber was wrapped with Cd foils (0·5 mm. thickness) and surrounded by paraffin;

(ii) the ionization chamber was kept within a lead enclosure of about 10 cm. thickness.

(*g*) No change in the number of spontaneous impulses per hour was detected, when a freshly prepared Po-source ($\sim 0{\cdot}2$ mc.) was placed between two of the plates of the ionization chamber. This precludes the possibility that the impulses might be due to the superposition of several α-particles.

(*h*) No evidence of spontaneous impulses was detected when the condenser plates were coated with oxides of some elements other than Uranium. This eliminates the possibility of any accidental discharge on the surface of an oxide film.

On the basis of the above control experiments, it can be reasonably inferred that the observed spontaneous impulses are due to the heavily-ionizing recoil fission products of Uranium. It is not likely that the observed effect may be associated with cosmic ray neutrons for the following reasons:—

(1) The control experiment (*f*) gives evidence to the contrary.

(2) The observed effect is equivalent to a current of 5 neutrons per sec. per sq. cm. if the cross-section of the fission of Uranium nucleus by neutrons is taken as 3×10^{-25} sq. cm.

This is calculated from the equation

$$N = n\nu\sigma$$

where N = number of fission impulses per sq. cm. of thin surface layer per sec.

n = number of neutrons responsible for the observed effect.

ν = number of atoms of the kind involved per sq. cm. of surface.

σ = cross-section for fission by neutrons.

Such a high concentration of particles per sq. cm. per sec. is not found even in the case of electrons (at sea-level), the most abundant constituent of cosmic rays. In fact, the flux of ionizing particles at sea-level, according to Street and Woodward (1934), is 1·48 per sq. cm. per min., while the corresponding neutron flux, at sea-level, according to Montgomery and Montgomery (1939) is only $0{\cdot}091\pm0{\cdot}007$ per sq. cm. per min.

B. *Determination of the half-life period of the spontaneous fission of the Uranium nucleus*

In spite of the usual precautions, the above experimental arrangement, was still susceptible to external mechanical disturbances. Most of the observations had therefore to be taken during the night, when such disturbances were reduced to a minimum. Besides, on account of its high amplification factor, the proportional amplifier had a tendency to become capricious whenever slight departure from optimum operating condition was imposed by long and continuous working hours. A new method of detecting fission pulses was therefore developed which is almost equally efficient but more stable than the previous arrangement. Immune to external disturbances, it could be operated, unattended, practically for any length of time.

Principle of the method

The underlying principle is that of proportional counter action. It is known that if the potential across a counter is sufficiently low so that it lies in the Region C of Fig. 2A,

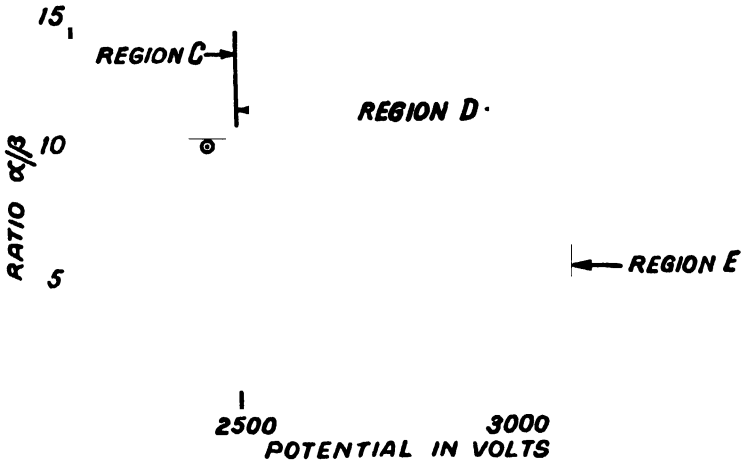

Fig. 2A.

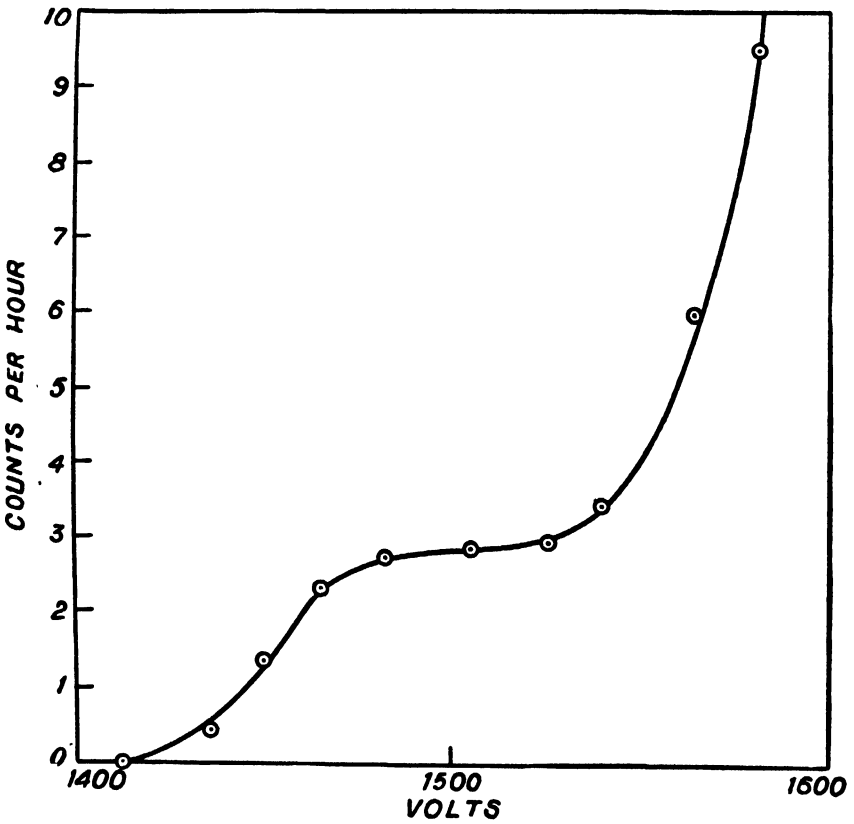

Fig. 2B.

the voltage pulse is proportional to the initial number of ions. If the voltage is so high as to fall in Region D, the voltage change of the wire will not be proportional to the initial number of ions, the departure from linearity being greater, the greater number of ions formed. These relations were experimentally verified by Korff (1940). Using a BF_3 counter in the C region, he shot α-particles of known range through a thin glass window and measured the amplitude of the output pulse. Next, he exposed the counter to intense γ radiation from radium. This radiation produced β-particles within the counter which liberated various number of ion pairs. The maximum amplitude of the output pulse would be produced by a β-particle whose range was just equal to the maximum path length within the sensitive volume of the counter. The ratio of the alpha-particle ionization pulse to that due to the maximum β-ionization pulse was found to be about 10·7, in agreement with theoretical calculation. As the potential of the counter was increased to Region D, the size of the pulses remained unequal, but their ratio decreased with increasing voltage. Finally, when the potential was so increased that the G-M Region E was reached, the pulses resulting from α- and β-particles became equal. Fig. 2A shows the actual observations.

Using a U_3O_8-lined counter, we have found that the relationships illustrated in Fig. 2A substantially hold good even when the fission pulses are taken into account. For example, in Region E the pulses due to β, α and ϕ (fission) are equal. As the potential of the counter is lowered into Region D, the pulses become unequal. The ratios ϕ/α and α/β increase with decreasing potential. When the potential is lowered still further, the proportional Region C is entered and the size-ratio of the pulses become independent of the counter potential. Now, at the threshold of the proportional region, the voltage-pulse due to a β-particle would be extremely small and inconsequential, whereas both α- and ϕ-particles would produce sufficiently large ionization pulses to trip a relatively low-sensitivity proportional amplifier. The range of the α- and ϕ-particles from Uranium being roughly equal, the former produces a much larger number of ion pairs for the same length of path. In order, therefore, to detect fission particles in the presence of alpha particles, one can proceed in two different ways: either (1) by reducing the gain of the amplifier so that an input voltage higher than that produced by α-particles is required to register a count, or (2) by reducing the gas-amplification of the proportional counter by lowering the counter potential to such an extent that the ionization pulse due to α-particles falls below the sensitivity limit of the amplifier.

In the following investigation, we have chosen the second method for its being easier in manipulation and more readily amenable to quantitative measurement.

Measurement

The Uranium oxide (U_3O_8) was finely powdered in an agate mortar under ethyl alcohol until a suspension was formed. The alcohol-suspended U_3O_8 was slowly poured on the inside of a cylindrical brass tube transferring a small portion at a time and evaporating the alcohol very slowly. The layer had a thickness of $\sim$5 mg./cm.^{2} It was found that too thin a layer reduced the counting rate per hour, while thicker layers ceased to increase the counting rate due to the absorption of fission particles by the material. The two open ends of the brass tube were closed with ebonite discs, fitted with amber plugs, earthed guard rings and carrying a central tungsten wire as shown in Fig. 3. The tube was evacuated and filled with dry CH_4 at a total pressure of 0·1 atmosphere.

The low pressure of the counter tube incidentally reduced:

(1) the working potential of the proportional counter, and

(2) the ionization current due to the α-particles of U. thus making the operational conditions more satisfactory.

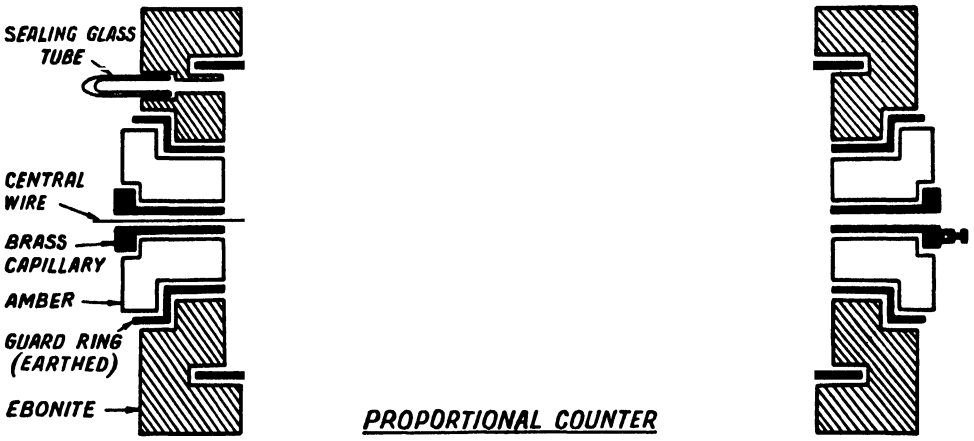

FIG. 3.

The counter was connected with a low-sensitivity and stable proportional amplifier. The stabilized H.T. was connected to the central wire, while the body of the tube was directly connected to the grid of the first tube of the proportional amplifier. In order to avoid the superposition of the individual α-particle pulses of U, the grid-leak of the first tube was made only 0·5MΩ. The counter potential was now gradually lowered till the α-particles were no longer registered. The counting rate of such a counter tube as a function of the applied potential is shown in Fig. 2B. The appropriate working potential for the counter tube for counting natural fission pulses was found out in the following way:—

A (Ra–Be) neutron source was kept near the counter tube, while gradually lowering the applied potential. It was found that the number of impulses per hour dropped sharply, then remained practically constant over a small plateau (range ~40 volts) and then declined gradually to zero. The impulses registered while the counter potential passed along the plateau was evidently due to the neutron induced fission pulses. While counting the natural fission pulses, the potential corresponding to the mid-point of the plateau was given to the counter. Because of the random emission of fission-particles with time and the small number emitted per hour, the period of observation was made about 1,200 hours.

CALCULATION

The number of ϕ-particles counted per hour is not the rate of emission from Uranium, since the thickness of the U_3O_8 layer is necessarily finite. Some of the ϕ-particles will fail to reach the surface of the layer or fail to travel the minimum distance in the counter necessary for detection. Again, many particles will travel in a direction away from the sensitive volume of the counter and fail to be registered. This missing number of particles must be taken into account for the accurate estimation of the half-life period of spontaneous fission.

Urry (1941) was confronted with a similar problem in connection with the estimation of small amounts of U by counting the number of α-particles emitted per hour by a flat thin layer of an Uranium-containing material. For a thin layer, he observed that the α-particles counted per hour could be expressed by the relation

$$c = CB(1-Sm), \quad .. \quad .. \quad .. \quad .. \quad (1)$$

where c = the mean number of α-particles counted per hour;

C = the mean number of α-particles emitted per hour in all directions, from Uranium in a perfectly thin layer;

m = weight of a thin layer;

B = the ratio of the α-particles counted to the number which would be counted in all directions;

S = a parameter which is a function of R, r, A, μ and d;

R = the mean range of α-particle in the gas of the ionization chamber;

r = the minimum distance that an α-particle must travel above the layer for detection. This is determined from the circuit characteristics of the apparatus;

A = Area of the thin layer;

$\frac{1}{\mu}$ = the stopping power of the thin layer for α-particles;

d = the density of the thin layer.

The value of B for a thin layer on a flat dish is evidently 0·50. For a cylindrical counter, the internal surface of which is coated with a thin layer of U_3O_8, it can be shown (see Appendix) that the value of B is reduced to $0{\cdot}50-\frac{1}{8}\left(\frac{r}{R}\right)$,

where r = smallest path-length of the α-particle to be capable of detection;

2R = diameter of the counter tube.

The equation (1) can be readily adopted for the case when ϕ-particles are counted instead of α-particles as stated above, with one important modification. Since the fission process involves the simultaneous emission of two particles in opposite directions, it is obvious that the value of B during the counting of ϕ-particles may be taken to be twice that calculated for the α-particle counting if we represent each single count by an individual fission process.

We thus have

$$c = 2CB(1-Sm) \quad .. \quad .. \quad .. \quad (2)$$

An independent value of Sm for thin layers is not easily determinable. One can, however, eliminate the term by measuring values of c for two or more values of m. Using a number of thin layers, we have found experimentally that the correction due to the factor (Sm) is really small. We have, therefore, provisionally neglected it and based our calculation on the simplified equation

$$c = 2CB \quad .. \quad .. \quad .. \quad (3)$$

We have seen that

$$B = 0{\cdot}50-\frac{1}{8}\left(\frac{r}{R}\right) \quad .. \quad .. \quad .. \quad (4)$$

So the next problem is: What value should be adopted for r, the minimum path-length of the ϕ-particle within the sensitive volume of the counter so that it is registered?

The energies of the fission particles have been estimated from the ionization produced by them. All of these estimates are based on measurements which give the number of pairs of ions produced by fission fragments. In order to calculate the energy it is necessary to assume a mean energy per pair of ions. This is usually taken to be the same as the value which is known from experiments with α-particles. Now, Kanner and Barschall (1939) have obtained a number energy curve with two fairly sharp peaks at energies of 65 and 97 eMV. The maximum energy of α-particles emitted by Uranium II is 4·4 eMV. The range of ϕ-particles as given by Joliot (1939), Corson and Thornton (1939) is ~3 cms., while the maximum range of α-particles emitted by Uranium II in air at N.T.P. is 2·90 cms. The ranges of the α- and ϕ-particles being thus practically equal, the size-ratio ϕ/α is ~15 in the C region of Fig. 2A. The setting of our apparatus being such that α-particles are not counted, while only the ϕ-particles are counted, we can assume that only those ϕ-particles having a path length of more than 0·06 × maximum range of α-particles will be counted. Now, in the fission-counter, filled with CH_4 at 0·1 atmospheric pressure, the maximum path-length of α-particles can be as long as ~25 cm., the approximate length of the counter tube. We can therefore reasonably assume that only those ϕ-particles having a length greater than $(25 \times 0{\cdot}06) = 1{\cdot}5$ cm. within the sensitive volume of the counter will be registered.

Taking the value of $r = 1{\cdot}5$ cm.,

we have $c = 2C\left[0{\cdot}50 - \frac{1}{8}\left(\frac{1{\cdot}5}{2{\cdot}5}\right)\right]$

$= 0{\cdot}85C.$

Now, if C be the actual number of Uranium nuclei undergoing spontaneous fission per hour, while m the mass of Uranium contained in the thin layer of U_3O_8, then the decay constant for spontaneous fission is given by

$$\lambda_f = \frac{C \times 24 \times 635}{m \times 2{\cdot}52 \times 10^{21}} \text{ year}^{-1}$$

Since 1 gm. of U contains $2{\cdot}52 \times 10^{21}$ atoms.

The half-life period for spontaneous fission is given by

$$T_f = \frac{0{\cdot}693}{\lambda_f} \text{ years.}$$

Results

The experimental results are presented in the following table:—

Wt. of U_3O_8 layer in gm.	Wt. of Uranium in the layer in gm.	Number of ϕ-particles per hour	Total number of ϕ-particles emitted per hour $C = \frac{c}{0{\cdot}85}$	Remarks
2·48	2·10	$2{\cdot}73 \pm 0{\cdot}02$	$3{\cdot}21 \pm 0{\cdot}03$	

Assuming that U^{238}, the most abundant isotope of Uranium (99·2%) undergoes spontaneous fission, the corresponding half-life period,

$T_f = 1{\cdot}3 \times 10^{16}$ years.

Discussion of the result

Uranium is a mixture of three isotopes U I (U^{238}), U II (U^{234}) and U^{235}. In the ordinary mixed material, their percentages are 99·28%, 0·006% and 0·71% respectively. It is not yet known which isotope of Uranium is responsible for spontaneous fission.

The phenomenon of spontaneous fission is attributed to the non-classical penetration of the potential barrier. In fact, there is a small but finite probability for spontaneous fission of all nuclei which can divide exothermically. Since U^{235} undergoes fission when irradiated with thermal neutrons, whereas U^{238} requires higher energy neutrons for the same purpose, it is likely that the former can undergo spontaneous fission more readily than U^{238}. It would be interesting to search for the spontaneous fission in each of the separated isotopes of Uranium.

There is again the likelihood that Uranium does not itself undergo spontaneous fission, but one of its disintegration product does. For example, Uranium I emits α-particle and is transformed into UX_1:

$$UI \xrightarrow{\alpha} UX_1$$

In order to ascertain whether UI or UX_1 is responsible for spontaneous fission, Petrzhak and Flerov mixed with U_3O_8 layers about 12 times more UX_1 than that contained in Uranium itself. No increase in effect being observed, it was concluded that the spontaneous fission cannot be attributed to the daughter element UX_1.

Further, since the conditions for the emission of α-particles and β-particles are independent of each other, it is not surprising that they are often both satisfied for the same nucleus and lead to branching, as with C-products. The necessary condition for such branching is that the two probabilities for disintegration be of nearly the same order of magnitude so that an appreciable fraction of the nuclei will disintegrate in the less probable way. Thus, M. Perey (1939) has shown that Actinium which has hitherto been known as only β-active, is also slightly α-active. The same consideration may perhaps also apply to Uranium. The latter, although essentially known as only α-active, may in addition, be slightly β-active. If so, the atomic number of the corresponding daughter nuclei would be 93, when spontaneous fission would be more probable. We are testing this point by separating this transuranic element as $_{93}$eka Re (Neptunium) or even as $_{94}$eka Os (Plutonium). These results will shortly be reported elsewhere.

The author expresses his grateful thanks to Prof. D M. Bose, Director of the Bose Research Institute, for his keen interest and inspiring suggestions. His thanks are also due to Prof. N. R. Sen for his kind help in the calculation of the reduction factor.

Appendix

Calculation of the Reduction factor 'B' for α-rays in the case of a circular cylindrical counter tube whose internal surface is coated with a perfectly thin layer of U_3O_8.

The mathematical problem can be formulated thus:

Given a perfectly thin and uniform layer of Uranium on the inner surface of the cylindrical tube, α-particles will emanate from every point of that layer and fly in all possible directions. Of these particles, only those which are emitted inside the cylinder and at the same time cover a minimum distance P within it before coming into contact with the surface of the cylinder again, will produce sufficient ionization to be recorded by the experimental arrangement. Thus r is the minimum distance through which the emitted

α-particle must travel in order to be detected. The layer being uniform, we shall have to calculate only the fraction of the total number of particles emanating from a point on the cylinder which will cover a distance greater than r before touching the surface of the cylinder again.

Suppose O is the point in [Fig. (i)] from which α-particles emissions take place. We imagine a sphere of radius r described round O as centre. This sphere will cut the surface

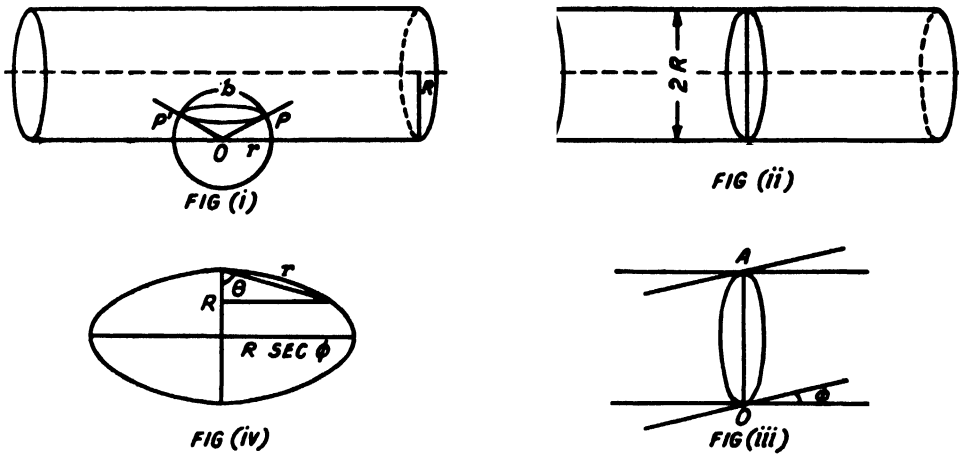

FIG. 4.

in a curve b. Now, if we draw a cone OPP' with O as vortex and b as basic curve, all lines drawn from O inside the cylinder and lying within the cone, have lengths greater than r, while the lines drawn outside the cone and within the cylinder will be less than r. Thus the α-particles emitted outside the cone will not be counted. So our object will be attained if we calculate the solid angle subtended by the curve b at the point O. We can calculate this solid angle subtended by the curve b at the point O. We can calculate this solid angle in the following manner:—

Consider the plane sections of the cylinder through OA.

There are two principal sections:

(1) A circle of radius R [AO in Fig. (ii)].

(2) A pair of straight lines, of radius of curvature ∞—[AB and the parallel line through O in Fig. (ii)].

Any other normal section through OA [as in Fig. (iv)] will be an ellipse. If the plane of this section makes an angle ϕ with the generator through O, the major axis of the ellipse will be R sec ϕ, and the minor axis R [Fig. (iv)]. From the point O and end of minor axis, we draw a chord of the ellipse of length r, making an angle θ with the minor axis [Fig. (iii)]. This chord of length r will lie on the cone in question.

The required solid angle is then

$$I = \int_0^{2\pi} \int_0^{\theta} \sin\theta \, d\theta \cdot d\phi = \int_0^{2\pi} (1 - \cos\theta) \, d\phi \cdot$$

$$= 2\pi - \int^{2\pi} \cos\theta \cdot d\phi.$$

In order to calculate the above integral we refer to the geometry of the ellipse. From the equation of the ellipse, we have

$$\frac{r^2 \sin^2\theta}{R^2 \sec^2\phi} + \frac{(R - r\cos\theta)^2}{R^2} = 1$$

giving the following equation for $\cos\theta$

$$r\cos^2\theta \sin^2\phi - 2R\cos\theta + r\cos^2\phi = 0$$

from which we get

$$\cos\theta = \frac{R}{r}\sec^2\phi \pm \frac{R}{r}\sec^2\phi\left[1 - \frac{r^2}{R}\cos^2\phi \sin^2\phi\right]^{\frac{1}{2}}.$$

$$\left(\frac{r}{R} < 1\right)$$

Since $\cos\theta \leq 1$, we have

$$\cos\theta = \left[\frac{1}{2}\cdot\frac{r}{R}\sin^2\phi + \frac{1}{8}\frac{r^3}{R^3}\cos^2\phi \sin^4\phi + \cdots\right].$$

Hence the integral

$$\int_0^{2\pi} \cos\theta . d\phi = \int_0^{2\pi}\left[\frac{1}{2}\cdot\frac{r}{R}\sin^2\phi + \frac{1}{8}\frac{r^3}{R^3}\cos^2\phi \sin^4\phi + \cdots\right]d\phi.$$

On integration, we get,

$$I = 2\pi - \frac{\pi}{2}\left(\frac{r}{R}\right)\left[1 + \frac{1}{2^5}\cdot\frac{r^2}{R^2} + \cdots\right].$$

Hence the reduction factor B is

$$\frac{I}{4\pi} = \frac{1}{2} - \frac{1}{8}\left(\frac{r}{R}\right)\left[1 + \frac{1}{2^5}\left(\frac{r}{R}\right)^2 + \cdots\right] = 0{\cdot}50 - \frac{1}{8}\left(\frac{r}{R}\right) \text{ approximately.}$$

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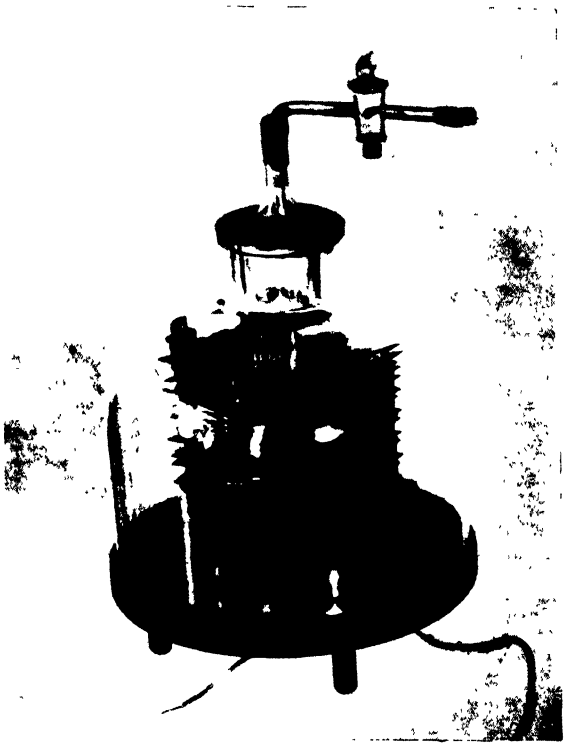

FIG 1A Ionization chamber.

FIG 1B. Neutron-induced fission pulse of U.

FIG. 1C Spontaneous fission pulse of U.

VIII. VERNALIZATION OF INDIAN CROPS

II. Photostage in Wheat (Triticum vulgare) and Oat (Avena sp.)

By B. K. Kar

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In a previous communication [1] it has been reported that pre-sowing cold treatment (vernalization) though not alone effective in enhancing the ear-emergence in the varieties I.P. 165; I.P. 52 and I.P. 4 yet its importance and utility in plant growth and development is beyond question for the fact, that pre-sowing cold treatment favours: (i) an increase in green pigment in the young seedlings [2], (ii) a greater output of tiller formation, and (iii) a higher percentage of culm to tiller formation with consequent higher yield. The ineffectiveness of the pre-sowing cold temp. treatment in the matter of earliness of flowering has been sought for in the subsequent photophase. In this connection it has been found possible to induce a significant earliness in case of vernalized seedlings when grown for a certain period under long-day conditions. Even the exposure to long days (12 hours to continuous days) for a period of 15 days at the early stage induces an earliness of 10–15 days. This finding is in agreement with that noted by Meljnik (Lysenko, 1932E) in experiments with vernalized and unvernalized winter wheat grown at a high temp. In a 10-hour or continuous day, only the vernalized plants grown in continuous day headed (Whyte[3]). In case of these Indian wheat varieties long day or continuous day cultures attain earliness irrespective of vernalization process and similar results were reported by Pal and Murty.[4] This behaviour clearly indicates the difference in nature of these Indian grown wheat varieties from that of temperate or hard winter varieties, and brings out into prominence the importance of required post-sowing day lengths.

Some preliminary observations on this aspect have already been reported [5] and an attempt has been made to explain the difference in behaviour of these varieties of wheat from that of the temperate varieties in the matter of earliness of ear-emergence, on the availability of the required day lengths. In the following paper the detailed results on the development of the photostage, its after-effects on the time of ear-emergence and its relation with the phasic development of wheat and oat seedlings have been given.

Material and Method.—Two varieties of wheat I.P. 4 and I.P. 52 and a variety of oat I.P. 1 were vernalized in cold (4°–6°C.) for 21 days and then sown in tubs. As soon as the seeds sprouted the tubs were separated in two groups. The first group was always kept as a control, i.e. allowed to grow under ordinary day and night conditions and from the second group eight tubs containing five plants each were transferred each week to long days (20 hours light period—daylight supplemented with artificial illumination during night—four 100-watt lamps at a distance of 3 ft. from plants, thus one lamp illuminated two tubs) beginning from the day of sprouting to the time of ear-emergence. They were kept under long-day conditions for a period of 7 days or 15 days and then allowed to grow and develop under ordinary day and night conditions. The experiments were repeated with the crops of three different sowings in case of wheat varieties and with only one sowing of the oat variety. Simultaneous experiments were also conducted with short-day photoperiods (8 hours) at different stages of the seedlings, as was done in case of

long-day series. The seedlings were kept in sunlight for 8 hours in the morning and then transferred to a darkroom, and again in the night they were kept in the open.

Experimental Results

Long-day series:—

The first sowing was done on November 6, 1942. The seeds sprouted after two days and the first set was transferred to long-day conditions on the 5th day from sowing and the age of the seedlings were also taken from the sowing date. The other sets were transferred at the ages of 13, 20 and 27th days. The results obtained have been summarized in Table I.

Table I

Sowing date, 6th November, 1942, I.P. 4. (Long-day period.) Average of 40 Plants

Age from sowing in days	Period of long-day treatment	Ear-emergence from sowing	Earliness over control	Yield in gm.
5	7 days	36·2 days	17·8 days	158·0
5	15 ,,	30·4 ,,	23·6 ,,	135·5
13	7 ,,	38·7 ,,	15·3 ,,	156·6
13	15 ,,	32·5 ,,	21·5 ,,	134·6
20	15 ,,	38·0 ,,	16·0 ,,	159·0
27	15 ,,	40·0 ,,	14·0 ,,	160·0
Control	....	54·0 ,,	....	169·5

The effect of long-day treatment (20 hours light period) on ear-emergence at different ages of the seedlings.

Table II

Sowing date, 6th November, 1942, I.P. 52

I.P. 52.

Age from sowing in days	Period of long-day treatment	Ear-emergence from sowing date	Earliness over contol	Yield in gm.
5	7 days	48·2 days	9·8 days	158·0
5	15 ,,	34·5 ,,	23·5 ,,	146·0
13	7 ,,	48·6 ,,	9·4 ,,	159·15
13	15 ,,	38·7 ,,	19·3 ,,	170·9
20	15 ,,	42·1 ,,	15·9 ,,	166·6
27	15 ,,	47·0 ,,	11·0 ,,	178·5
Control	....	58·0 ,,	....	....

The effect of long-day treatment (20 hours light period) on ear-emergence at different ages of the plants. Average of 40 plants.

It was found that with the availability of long-day conditions at the early seedling stage of 5–13 days age a significant earliness in ear-emergence was obtained. Both the varieties responded similarly. Even an exposure to long-day conditions for a short period of 15 days only was sufficient to induce an earliness of 24 days in both the varieties. As the seedlings pass through their developmental stages, the effect of the same long-day conditions become less and less effective in inducing earliness. The maximum earliness

as a result of exposure to a period of 15 long days was obtained in the early stage of 5–13 days age. In later stages, i.e. 20 or 27 days old seedlings, the same degree of earliness was not obtained, but the exposure to long-day conditions was at the same time not ineffective. It was, therefore, seen that photostage in these wheat varieties was effective in the early stages of the plant beginning from just after sprouting to that of 15 days age and a long-day treatment confined to this developmental phase was quite effective in inducing a significant earliness. In control plants this developmental stage (photostage) had to be passed under available natural short-day conditions (8–10 hours period) of the tropics and hence the delayed ear-emergence when compared to the seedlings cultured under long-day conditions. It was also seen that photo-induced earliness brought about a decrease in yield which varied under different treatments as compared to the normal seedlings.

The above results were repeated in a second set of experiments where the seedlings were exposed to long days of 7 and 15 days respectively at different ages, beginning from earliest to 27 days old seedlings. The results have been summarized in the Tables III, IV and V.

TABLE III

Sowing date, 13th November, 1942

I.P. 4.

Age from sowing in days	Period of long-day treatment	Ear-emergence from sowing date	Earliness over control
6	7 days	40·7 days	9·3 days
6	15 ,,	31·4 ,,	18·6 ,,
13	7 ,,	40·2 ,,	9·8 ,,
13	15 ,,	35·0 ,,	35·0 ,,
20	7 ,,	41·2 ,,	8·8 ,,
20	15 ,,	38·4 ,,	11·8 ,,
27	7 ,,	41·3 ,,	8·7 ,,
27	15 ,,	37·0 ,,	13·0 ,,
Control	....	50·0 ,,	....

The effect of long-day treatment on ear-emergence at different ages of the plants. Average of 40 plants.

TABLE IV

Sowing date, 13th November, 1942

I.P. 52.

Age from sowing in days	Period of long-day treatment	Ear-emergence from sowing date	Earliness over control
6	7 days	50·4 days	7·6 days
6	15 ,,	38·8 ,,	19·2 ,,
13	7 ,,	50·6 ,,	7·4 ,,
13	15 ,,	39·3 ,,	18·7 ,,
20	15 ,,	41·2 ,,	16·8 ,,
Control	....	58·0 ,,	....

The effect of long-day treatment on ear-emergence at different ages of the plants. Average of 40 plants.

TABLE V

Sowing date, 13th November, 1942

Age from sowing in days	Period of long-day treatment	Ear-emergence from sowing date	Earliness over control
I.P. 4.			
15	7 days	42·7 days	6·3 days
15	15 „	38·2 „	10·8 „
22	7 „	44·4 „	4·6 „
22	26 „	39·0 „	10·0 „
29	20 „	43·0 „	6·0 „
Control	....	49·0 „	....
I.P. 52.			
15	7 days	48·4 days	8·2 days
15	15 „	42·3 „	14·3 „
22	7 „	48·7 „	7·9 „
22	26 „	43·2 „	13·4 „
29	20 „	45·5 „	11·1 „
Control	..	56·6 „	....

From the above tabulated results it was seen that earliness in ear-emergence was proportional to the number of long days the seedlings have received in the photostage. The earliness brought about as a result of 7 days' treatment was found to have doubled when the long-day treatment period was continued from 7 days to 15 days. The long days were most effective in inducing earliness when given at the early stage. On the other hand in seedlings of 22 days old, even under increased number of long-day conditions of 26 days (i.e. more than 15 days which was given in case of 5–15 days old seedlings), no corresponding enhanced earliness was noticed. The similar is the response in case of older seedlings of 29 days. It was therefore definitely indicated that to bring about a significant earliness in ear-emergence long-day period was essential in the photostage which in these wheat varieties was found to be very effective in young seedlings from sprouting to 15 days and in seedlings older than 15 days, the long-day periods were found to be also effective but to a lesser degree. Here it may be mentioned that during the exposure to long-day periods the seedlings on average have received a higher temperature varying from 2°–4°C. during night than the control sets. After the treatment to long-day periods both were kept under same light and temp. conditions throughout the rest of their life-cycle.

Short-day series :—

Simultaneously with the long-day photo-periodic treatments another set of experiments were run under short-day conditions. The seedlings were allowed to receive 8 hours light period in the morning from 7 a.m. to 3 p.m. and then transferred to the darkroom for the rest of the day. They were taken out again after dusk and kept in the open for the night. The number of days taken for the ears to emerge from the sheath as compared to those of controls, growing under normal day and night conditions in seedlings of different ages, have been given in Table VI.

TABLE VI

Sowing date, 13th November, 1942

I.P. 4.

Age from sowing in days	Short-day period of 8 hours	Ear-emergence from sowing date (in days)	Delayed from control (in days)
5	7 days	65·3	7·5
5	15 „	72·4	14·6
13	7 „	60·2	2·4
13	15 „	62·5	4·7
20	15 „	60·2	2·4
27	15 „	57·3	nil
Control	....	57·8	nil
I.P.52.			
5	7 days	77·2	11·2
5	15 „	86·5	20·5
13	7 „	68·8	2·8
13	15 „	70·3	4·3
20	15 „	68·9	2·9
27	15 „	67·6	1·6
Control	....	66·0	....

The effect of short-day treatment (8 hours light period) on ear-emergence at different ages of the seedlings. Average of 40 seedlings.

If the available light period was further curtailed during the post-sowing period it was found that the time of ear-emergence was considerably delayed from that of the control seedlings. The vegetative growth was luxuriant and a large number of tillers were formed but they failed to bolt in time. The results showed that short-day conditions in the earliest stage (6–18 days age) induced a maximum delaying effect. In later stages the effect was not so marked. It can, therefore, be concluded from the short-day series of investigations that the photo-induction was effective in the early stages as was found in case of long-day series of investigations. The required photo-period, which was found to be in the region of long days in case of these varieties—when made available at the photostage (as was done in long-day series of investigations)—then an earliness was induced; but if it was not available in nature or curtailed (as was done in short-day series of investigations) a delaying effect in ear-emergence resulted.

EXPERIMENTS WITH OAT

At progressive stages of the growth and development of oat seedlings definite periods of long-day conditions were given similar to those of the wheat seedlings. The earliness in ear-emergence over the control (growing under short-day conditions) is tabulated below in Table VII.

In oat also the most effective long-day periods were found to be in the region from 7 to 15 days old seedlings which was found to be similar to wheat seedlings. But the exposure to long-day periods at later stages up to 50 days age was also found to be effective. The exposure to a long-day period of 15 days at the age of 7th day seedling brought about

an earliness of 43 days nearly half from that of control. At a later stage even with increased numbers of long days, the corresponding earliness was not enhanced. The oat variety like wheat varieties completed its normal course of developmental stages leading to ear-emergence within 82 days and the effect of long days at the photostage was instrumental in completing those developmental stages of the life-cycle within 43 days.

TABLE VII

Sowing date, 13th November, 1942

Oat I.P. 1.

Age from sowing in days	Period of long-day treatment	Ear-emergence from sowing date	Earliness over control
7	7 days	51 days	31 days
7	15 „	39 „	43 „
15	15 „	39 „	43 „
23	7 „	52 „	30 „
23	26 „	45 „	37 „
30	19 „	49 „	33 „
50	30 „	66 „	16 „
Control	....	82 „	....

Effect of long-day treatment (20 hours light period) on the time of ear-emergence of different ages of the seedlings. Average of 40 seedlings.

It was reported before that this variety of oat did not respond to vernalization alone but vernalization together with post-sowing long-day conditions were effective in inducing a significant earliness.

DISCUSSION

In the matter of earliness of Indian cereals (wheat and oat) the results described above clearly indicated the importance and the necessity of certain number of long-day periods in order to hasten the development of the so-called photostage. In a previous communication the importance of thermal phase has been clearly shown. But the thermal phase alone without the proper post-sowing photostage was not effective in bringing a significant earliness. This further proved the importance of post-sowing environmental factors which had been stressed previously. As regards the age of the plant when the photostage was found to be most effective, it was found that the photostage followed the thermal phase with the sprouting of the seeds to an age of 20 days of the seedlings. But the long-day periods were found to be more or less effective in seedlings more than 20 days age, but not to that extent as in earliest stages of the seedlings. Here it may be mentioned that long-day treatments were accompanied with higher temperature of 2°–4°C. than the control seedlings which to some extent influenced the photostage. The effect found in seedlings of older age was partially due to the high temperature, which was needed at this advanced stage, and it was therefore difficult to limit and isolate the effects produced only by long days. But the importance of a definite period of light essential for the completion of certain developmental stages leading to the bolting of the tillers, was also made evident from the results of the short-day series of investigations, where the curtailment of the available light period resulted in considerable retardation

of the time of ear-emergence. The effect of earliness by photo-induction on yield was different under different periods of treatment. The yield per plant decreased with increased earliness and a minimum yield was obtained in case of maximum earliness from the normal seedlings.

The importance of long-day periods during photostage at present could only be stressed with certain limitations because the cereals also normally develop and flower under short-day conditions in India. But it was proved beyond doubt that even a limited number of long days at the proper photostage was effective in inducing a rapid rhythm of developmental phases than those growing under normal short-day conditions.

Summary

It was previously noted that pre-sowing cold treatment (vernalization phase) with post-sowing photostage of short-day lengths were not effective in bringing a significant earliness in ear-emergence in case of Indian cereals (wheat and oat). But by substituting long days in place of short days, a significant earliness sets in. This is further proved during this investigation on the following results:—

1. Wheat varieties (I.P. 4 and I.P. 52) when exposed to long days for a period of 15 days an earliness of 24 days was attained over the control.
2. The exposure to long days was most effective at the stage beginning from sprouting of the seedlings to 20 days age.
3. In oats (I.P. 1) similar treatment of long days brought about an earliness of 43 days over the control.
4. In these cereals, the photostage begins with the sprouting of the seeds and even a limited number of long days during the proper photostage were instrumental in bringing a significant earliness.
5. In the matter of flowering and the developmental stages leading to it, the short-day conditions of the tropics were tolerated, but availability of long days are more inducive to a rapid rhythm of the developmental phases.
6. The nature of these cereals with reference to thermal and photophases was found to differ from the temperate or hard winter cereals.

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IX. MANURIAL EXPERIMENTS ON JUTE

II. Effects of ammonium and nitrate nitrogen on the yield and the growth of the plant (Corchorus capsularis, Linn.)

By J. K. Choudhury

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In a previous communication, the results of a preliminary investigation with combinations of various chemical compounds on the yield and the rate of growth of one variety of the jute plant, viz. *C. capsularis*, Linn. (D. 154), were reported and it was then found that with the quantities used, cow-dung as a manure, gave the most significant yield of fibre, being closely followed by a mixture of calcium phosphate, ammonium sulphate and potassium chloride, and that there existed a general relationship between the height of the plant and its yield.[1] The present investigation, however, was undertaken in order to determine the effects of individual chemicals on the yield and growth of the plant, instead of employing a mixture of them as in the previous case and it was decided to observe, in the first instance, the effects of only the nitrogenous manures, such as ammonium sulphate and sodium nitrate, and to compare their results with those obtained by manuring with cow-dung only; and also to decide upon the most adequate quantities of these manures. It will be evident from the results reported here that there is a vast possibility for the employment of inorganic substances as manures in jute fields with economic advantages. A short review of some of the works carried out in this line have already been made in the previous communication [1] and need not be repeated here; a few more recent observations may be referred to in due course.

Lay-out and the Procedure

The lay-out of the design of the experiment is shown in Fig. 1, and the procedure followed was similar to that reported before.[1] It was a randomized block lay-out with rectangular plots, measuring 12 ft. by 6 ft. each and in them were tried ten treatments (including the control) with three replications for each of them. Treatments (except the untreated control) were cow-dung in three doses of 5, 10 and 15 seers and commercial ammonium sulphate and sodium nitrate of 1, 2 and 3 lbs. each and every plot received only one dose of each of these treatments. Seeds were sown thrice in the course of more than a month, but the first two attempts having proved unsatisfactory due to intense heat and absence of rains, the third attempt was made with success on the 7th June, 1943. Weekly height records of the ten standard plants per plot (selected at random) were kept from the 28th June, at the end of the third week from the date of sowing, to the 13th Sept., the fourteenth week, when the plants were ready for harvest. All the plants in a plot were then counted and measured before being put into water for retting. This experiment also, like the former one,[1] was carried out at the Bose Institute's Experimental Station at Falta in the 24-Parganas, Bengal, with the same variety of jute plant (viz. *C. capsularis*, D. 154) grown from seeds obtained from the laboratories of the Central Jute Committee at Dacca.

A	C	H	G	E	M	L	B	F	D
H	D	L	B	F	G	A	M	E	C
L	A	E	C	M	H	D	F	G	B

FIG. 1. Lay-out of the plots: A—Control (untreated); B—Cow-dung, 5 srs.; C—Ammonium sulphate, 1 lb.; D—Sodium nitrate, 1 lb.; E—Cow-dung, 10 srs.; F—Ammonium sulphate, 2 lbs.; G—Sodium nitrate, 2 lbs.; H—Cow-dung, 15 srs.; L—Ammonium sulphate, 3 lbs.; M—Sodium nitrate, 3 lbs.

EXPERIMENTAL RESULTS

A. *Yield of fibre*

Jute fibres obtained from all the plants growing in the plots were collected separately, dried in the sun according to usual practice and their dry weights recorded. The results are given below in Table I and they are expressed in lbs. per one hundred plants from each plot. From the analysis of variance included in Table II, it will be found that although the treatment effect is significant at 1% level, the block effect is negligible. As regards the order of effectiveness of the various treatments on the yield, treatment L in this respect is the most effective of them all. Next to L is M and then F, but L and M do not differ significantly, so also M and F. L, however, is significantly different from F at the verge of 1% level (L~F = 0·4700) though not strictly at that level. After F comes G, but their difference again is significant at the 5% level only. Other treatments are more or less of the same order of effectiveness and it is interesting to note that the three doses of cow-dung in treatments E, B and H are found to be equally effective.

TABLE I

Yield of jute fibre in lbs. per 100 plants in plots under different treatments

Treatment	Dung at doses			Am. sulph. at doses			Nitrate at doses			Control	Total
	1	2	3	1	2	3	1	2	3		
Block	B	E	H	C	F	L	D	G	M	A	
I	1·06	0·85	0·82	0·94	1·63	2·33	1·37	1·60	1·97	0·59	13·16
II	0·97	1·29	0·93	1·39	2·26	2·08	1·30	1·33	1·91	0·73	14·19
III	1·03	1·17	1·13	1·72	1·69	2·58	1·52	1·51	2·20	0·68	15·23
Total ..	3·06	3·31	2·88	4·05	5·58	6·99	4·19	4·44	6·08	2·00	42·58
Mean ..	1·02	1·10	0·96	1·35	1·86	2·33	1·40	1·48	2·03	0·67	

TABLE II

Analysis of variance (yield of fibre)

Due to	Degrees of freedom	Sum of squares	Variance	Ratio of variance
Block	2	0·2143	0·1072	2·62
Treatment ..	9	7·3145	0·8127	19·87**
Error	18	0·7362	0·0409	
TOTAL ..	29	8·2650		

Critical difference at 5% level—0·3465

" " at 1% level—0·4752

** indicates that the particular effect is significant at 1% level.

TABLE III

Mean weekly height in inches of the jute plants in different treatments

Treatments Weeks	A	B	C	D	E	F	G	H	L	M
III	10·77	13·38	14·78	11·08	12·17	17·23	14·23	13·60	15·85	14·23
IV	16·47	20·83	22·32	18 90	22·25	26·65	24·23	22·13	26·82	26·60
V	22·59	29·73	32·94	28·43	31·92	38·15	35·02	30·22	38·20	39·63
VI	29·80	40·20	45·40	41·00	43·37	52·33	47·17	40·50	51·53	53·63
VII	32·80	44·64	50·53	47·08	49·33	60·42	54·32	46·97	59·55	60·78
VIII	38·67	48·40	60·40	55·85	57·35	70·30	64·17	54·98	69·82	71·43
IX	44·03	55·97	69·27	63·43	66·30	79·88	73·25	62 77	80·25	82·80
X	50·41	63·15	77·45	71·88	73·78	88·72	81·33	68·45	89·17	92·88
XI	..	..	..	..	..	..	..	..	..	..
XII	61·32	77·37	92·82	90·00	89·33	107·07	99·10	81·37	109·73	114·37
XIII	65·60	83·93	101·23	96·87	97·20	114·90	105·73	89·30	117·13	122·07
XIV	69·07	87·60	107·60	101·83	102·50	121·47	111·30	95·63	121·97	127·10

B. *Growth records of the plants*

The growth in height of the ten standard plants (from the soil to the growing tip) per plot, selected earlier at random, were recorded for twelve weeks (except at the eleventh week which was missed unavoidably). Flowering began at about the tenth week in some plots and continued till the end of the experimental period when a considerable number of plants showed branching at their apices. Weekly average heights of the standard plants in different treatments are given in Table III, where each of the figures represents the average height of thirty plants (block differences having been found to be insignificant). Growth records of these plants at the thirteenth week are given in Table IV and their analysis of variance in Table V. This particular week is selected owing to its closeness to the harvest when the plants were fully mature, but similar results may also be obtained at any other stage of the plants.

TABLE IV

Total height in inches of the ten standard plants in different blocks and under different treatments at the thirteenth week

Treatment Block	A	B	C	D	E	F	G	H	L	M	Total
I	526	1034	915	994	984	1125	1203	1001	1240	1220	10242
II	765	891	1016	910	1076	1236	1104	789	1106	1266	10159
III	677	593	1106	1002	856	1086	865	889	1168	1176	9418
Total ..	1968	2518	3037	2906	2916	3447	3172	2679	3514	3662	29819
Mean ..	656	839·3	1012·3	968·7	972	1149	1057·3	893	1171·3	1220·7	

TABLE V

Analysis of variance (height at the thirteenth week)

Due to	Degrees of freedom	Sum of squares	Variance	Ratio of variance
Block ..	2	31164·87	15582·44	1·09
Treatment ..	9	782068·97	86896·55	6·13**
Error ..	18	255217·13	14178·74	
TOTAL ..	29	1068450·97		

Critical difference at 5% level—204·1
„ „ at 1% level—280·0

The analysis of variance in Table V shows that the treatment effect on the height of the plants is also significant at 1% level, whereas the block effect is negligible. Of the treatments, M is, however, found to be most effective in the present case followed by L, F, G, C, etc. But the effect of M does not differ from that of L, F and G at 5% level, although M is definitely better than any of the rest of the treatments. Here also there is little difference in the effects of the treatments E, B and H, but as in the case of the yields all of them are better than the untreated control in different degrees.

C. *Fresh and dry weights of the different parts of the plants under various treatments and the area of their leaves*

The effectiveness of some of these treatments became visible in the plants as early as in the fourth week of their growth and it became fairly established later in a week or two. Thus, at the fifth week some notes and measurements were taken as to the colour of the leaves, their size, general condition of growth of the plants in the plots, etc., and it was found that the treatments L, M and G particularly were already very promising. This has since been borne out by later observations and data we have already considered and given in tables. Here, in support of this fact, the results of some observations are given in Table VI to show the state the plants were in shortly after the close of the seventh week. Three plants, approximately of the same average height with that of the

TABLE VI

Mean fresh and dry weights (gms.) of leaf, bark and wood and area (sq. cm.) of leaf of plants grown under different treatments shortly after the end of the seventh week

	Leaf			Bark		Wood		
	Per plant (5 lvs.)		Per leaf	Per plant		Per plant		Av. height
Treatments	Fr. wt.	Dry wt.	Area	Fr. wt.	Dry wt.	Fr. wt.	Dry wt.	(inches)
A	2·22	0·41	29·64	6·28	1·20	9·07	1·30	37·50
B	2·98	0·45	39·67	9·94	1·77	15·67	1·97	43·78
C	4·67	0·76	56·55	16·33	2·78	27·22	3·24	51·88
D	4·06	0·71	49·52	14·81	2·59	24·22	2·96	49·65
E	2·91	0·67	49·45	15·78	2·86	22·72	3·14	52·43
F	5·72	0·93	69·28	28·22	4·62	45·67	5·31	61·48
G	5·06	0·89	59·62	26·38	4·52	39·89	5·12	56·48
H	3·63	0·63	44·77	14·67	2·65	21·72	2·87	49·33
L	7·40	1·22	83·45	36·67	5·69	62·33	6·72	62·77
M	6·50	1·07	77·05	33·17	5·42	53·94	6·01	61·40

standard plants, were selected at random from each plot. Their individual measurements in length were taken before separating their bark from the wood and tracing the area of the five fully expanded leaves from usually the fifth leaf at the apex to the ninth leaf down the stem. These operations were carried out as quickly as possible to ensure minimum loss of moisture from the materials, which were then weighed separately for their fresh weights. They were then dried and brought down to Calcutta for final drying and desiccation before taking their dry weights. The area of the leaves were obtained by weighing their respective traces against the weight of a standard piece of the same tracing paper. Each of the results put in Table VI is, therefore, the average of these materials collected from nine plants in three plots with the same treatment. They are given in greater detail in Tables VII, IX and XI, and their statistical analysis in Tables VIII, X and XII respectively. In all these tables only the dry weights of the bark and the leaves and also the area of the leaves are considered and with the help of the critical differences mentioned in each case, the treatment effect will be found to be significant at 1% level in all the cases. These

TABLE VII

Mean dry weights of leaves in gms. under different treatments

Treatment Block	A	B	C	D	E	F	G	H	L	M	Total
I	0·077	0·096	0·131	0·123	0·127	0·165	0·234	0·132	0·254	0·224	1·563
II	0·073	0·120	0·167	0·115	0·165	0·205	0·179	0·100	0·250	0·230	1·604
III	0·099	0·078	0·157	0·191	0·113	0·190	0·119	0·147	0·231	0·187	1·512
Total ..	0·249	0·294	0·455	0·429	0·405	0·560	0·532	0·379	0·735	0·641	4·679
Mean ..	0·083	0·098	0·152	0·143	0·135	0·187	0·177	0·126	0·245	0·214	

TABLE VIII

Analysis of variance (dry weights of leaves)

Due to	Degrees of freedom	Sum of squares	Variance	Ratio of variance
Block	2	0·00008	0·00004	0·36
Treatment ..	9	0·0669	0·0074	6·7**
Error	18	0·01922	0·0011	
TOTAL ..	29	0·08620		

Critical difference at 5% level—0·0567
„ „ at 1% level—0·0878

tables will also show that the order of effectiveness for the first four treatments is L, M, F and G in all these cases, but they usually do not differ from one another even at 5% level. The rest of the treatments, however, differ significantly at 1% level from the first two, viz., L and M. Similar results were also obtained with the fresh weights of these materials and also with fresh and dry weights of the wood (pith).

TABLE IX

Dry weights of barks of three plants in gms. under different treatments

Treatment Block	A	B	C	D	E	F	G	H	L	M	Total
I	2·16	6·22	5·74	5·56	7·68	12·54	21·84	9·94	19·45	18·06	109·19
II	3·99	7·10	7·80	7·09	10·90	16·17	12·18	6·72	14·85	17·55	104·35
III	4·67	2·66	11·45	10·68	7·17	12·92	6·65	7·16	16·95	13·22	93·53
Total ..	10·82	15·98	24·99	23·33	25·75	41·63	40·67	23·82	51·25	48·83	307·07
Mean ..	3·61	5·33	8·33	7·78	8·58	13·88	13·56	7·94	17·08	16·28	

TABLE X

Analysis of variance (dry weights of bark)

Due to	Degrees of freedom	Sum of squares	Variance	Ratio of variance
Block	2	12·85	6·425	0·59
Treatment ..	9	580·17	64·46	5·881**
Error	18	197·31	10·96	
TOTAL ..	29	790·33		

Critical difference at 5% level—5·678
„ „ at 1% level—7·786

TABLE XI

Mean area of leaves of plants under different treatments (sq. cm.)

Treatment Block	A	B	C	D	E	F	G	H	L	M	Total
I	31·72	44·36	53·25	44·32	48·29	62·43	78·02	49·98	94·27	80·93	587·57
II	27·89	46·30	62·19	40·76	62·72	79·21	60·15	33·75	85·34	90·86	589·17
III	29·35	28·35	54·22	63·45	37·34	66·19	40·69	50·58	70·74	59·38	500·29
Total ..	88·96	119·01	169·66	148·53	148·35	207·83	178·86	134·31	250·35	231·17	1677·03
Mean ..	29·65	39·67	56·55	49·51	49·45	69·27	59·62	44·77	83·45	77·06	

TABLE XII

Analysis of variance (area of leaves)

Due to	Degrees of freedom	Sum of squares	Variance	Ratio of variance
Block	2	517·34	258·67	2·13
Treatment ..	9	7675·26	852·81	7·012**
Error	18	2189·06	121·61	
TOTAL ..	29	10381·66		

Critical difference at 5% level—18·908
„ „ at 1% level—25·931

D. *Relation between the height of the plants and their yield*

The analysis of the height records of the standard plants and their yields (obtained separately from the total yield per plot) provided an opportunity to examine the relationship that may be existing between the two and from the data that are set out in Table XIII, this relationship is found to be distinctly in the positive (despite the treatment effects which have been overlooked) with a correlation coefficient of 0·81.

TABLE XIII

Mean height (in ft. at the 13th week) and yield (in lbs.) of the ten standard plants

Treatment	Height	Yield	Height	Yield	Height	Yield
A	4·3	0·0065	6·4	0·0048	5·6	0·0065
B	8·6	0·0130	7·4	0·0195	4·9	0·0048
C	7·6	0·0130	8·5	0·0130	9·2	0·0195
D	8·3	0·0130	7·6	0·0130	8·3	0·0195
E	8·2	0·0130	8·9	0·0130	7·1	0·0097
F	9·4	0·0230	10·3	0·0130	9·0	0·0230
G	10·0	0·0260	9·2	0·0260	7·2	0·0130
H	8·3	0·0130	6·6	0·0097	7·4	0·0097
L	10·3	0·0325	9·2	0·0195	9·7	0·0260
M	10·1	0·0195	10·5	0·0325	9·8	0·0260

Correlation coefficient—0·81

DISCUSSION

The effects of each treatment on the growth and the yield of the jute plants have been mentioned at length in the text as well as in the tables and it will be found that the first four places of preference are occupied by the treatments L, M, F and G, proving thereby that both ammonium sulphate and sodium nitrate had a significant effect on the growth of the plants as well as on their yield and in the quantities these two manures were employed, they were distinctly superior to the farmyard manure. It will also be noticed that while treatment M gave the best growth, treatment L gave the best yield, but their difference, as has been mentioned before, is not significant and this apparent contradiction may well be due to the little differences in the girth of the plants which have not been taken into consideration.[2] As regards other treatments with ammonium and nitrate, they were more or less comparable in their respective doses and one did not seem to be in any way decidedly superior to the other. Cow-dung, on the other hand, was found to be less effective than any of the treatments with ammonium or nitrate (except D on growth), both on the growth and the yield of the plants. An excess of dung in H was of no avail in this respect and was found to be actually less effective than E, with a smaller quantity of the same. In this connection some recent observations of the Indian Central Jute Committee may prove to be of interest. This Committee found that the application of 100 mds. of farmyard manure plus 10 mds. of lime gave a better yield of 8·1 mds. of fibre per acre compared to 7·5 and 7·0 mds. obtained by supplementing 3·6 and 1·8 mds. respectively of ammonium sulphate to the above treatment which was common to all of them.[3] Apart from the fact that these figures appear to be rather too low compared to the outturn the cultivators usually derive from an acre,[4] they will also be found to be different from those obtained by them in the previous year when an addition of ammonium sulphate had, however, a beneficial effect on the yield.[5] Our results, on the

other hand, show very clearly that the application of ammonium or nitrate as a manure had a marked effect both on the growth of the plants and their yield, and if they are to be expressed in terms of acreage, treatment L gave an outturn of about 32 mds. of fibre per acre and M more than 28 mds., whereas the untreated (control) and the dung-treated plots under A and E yielded only about 8 and 14 mds. respectively. Treatments F and G were also very effective and F, particularly, was only slightly inferior to M in this respect.

With regard to the growth in height of the plants and its relation to their yield, the results recorded in Table XIII confirm the observation made last year that there was a positive and direct relationship existing between the two, so that if there be an appreciable increase in height of the plants, in all probability there will also be a simultaneous increase in their yield.

Manuring with ammonium and nitrate not only brought about an increase in the height and the yield of the jute plants, but there seemed to be an all-round increase made in the weights of various parts of the plants, such as the leaf, the bark and the wood and also in the area of the leaf (Table VI). Similar observations have also been recorded with cotton,[6] sugar cane [7] and apple.[8] In cotton referred to above, it has been noticed that the size of the plant is largely a measure of the rate of nitrogen metabolism and the total growth of the plant depends primarily on the rate of development of the leaf surface. In sugar cane also an increased application of nitrogen was found to bring about an increase in leaf area and in the rate of stem elongation.[7]

Summary

1. A manurial experiment on jute plants (*C. capsularis*, D. 154) was carried out at Falta (24-Parganas, Bengal) to study the effects of ammonium sulphate, sodium nitrate and farmyard manures (cow-dung) in various quantities on the growth and yield of the plants.

2. Ammonium sulphate and sodium nitrate had a very marked effect on both the growth and the yield and increased the yield by twofold over that obtained from the dung-manured plots. An excess of dung was not of any additional advantage either on the yield or on the growth.

3. Both the ammonium and nitrate were almost equally effective on the growth of the plants and their yield.

4. There was also a positive correlation between the height and the yield.

5. Ammonium and nitrate increase the fresh and dry weights of the bark, the wood and the leaves of the plants and also the area of the leaves.

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X. STUDIES IN YEAST

I. Sporulation and Hybridization

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I. Introduction

The importance of yeast in the progress and development of the human race cannot be over-emphasized. Even during the very early times, these micro-organisms have been used to induce beneficial changes which would have been difficult to accomplish by other methods. As an example the yeast cells discovered in the bread along with the Egyptian Mummies may be cited. This is an indication that these ancient people knew at least some of the uses to which these organisms could be put to. Again, the Norsemen used to prepare an alcoholic drink from milk as is done today by certain Nomadic races—the fermentation of which was partly caused by yeasts. Today, we find the yeast of ever-increasing importance and interest. They are assuming great importance, in medicine, especially in relation to certain deficiency diseases, constipation and skin infections. In industry also, the yeast occupies a unique position. Without these organisms, baking, brewing, wine-making and distilleries will come to a standstill.

In these days of keen competition, it is essential to improve the quality of these industrial and medicinal products of yeast and the method usually adopted is by the addition of chemicals and accessory growth factors. But these methods not only increase the cost of production, but also complicates the process involved. Thus taking a cue from the significant results obtained by hybridization and selection in higher plants and animals, a few scientists have been endeavouring to apply the same methods to these micro-organisms, the pioneer in the field being Winge and Laustsen (1938, 1938*a* and 1939*b*). The result of their experiments will be dealt with briefly, later. Their methods now make it possible to undertake rational breeding work with yeast and the possibility to produce new yeast of commercial value. In passing, it may also be mentioned that as far as we are aware, no one else have succeeded in adapting these methods to breeding better types of yeast in India and hence we hope that our experiments recorded here will be of special interest to the industrialists of India.

In this connection the following quotation from Winge and Laustsen's (1939) publication is of special interest. They produced 14 new hybrid types of which 'one hybrid (No. 471) showed advantages to the parents, the production of dry matter and of carbon dioxide being particularly high, and it also sedimented satisfactorily. It is a matter of course this did not guarantee its value in practice. However, a British firm who produce baking yeast in several factors, received the hybrid, and after listing it they have praised it loudly and are now using it industrially instead of the yeast previously used. It appears justified to assume that a more comprehensive hybridization work planned for the special purpose of producing new yeast types of practical value, would lead to better and better results'.

II. Material and Methods

The present series of experiments are confined to the *Saccharomyces* and *Torulopsis* species and varieties, for the supply of which we are indebted to the Bengal Immunity Co., Ltd., and are listed below:

1. *Saccharomyces cerevisiae* var. B.I.
2. „ „ „ 2160
3. „ „ „ 918.
4. „ *Sake* „ 4134.
5. „ *ellipsoideus* var. 4108.
6. „ „ „ 4097.
7. *Torulopsis utilis* var. *major*—Y41.
8. „ „ „ 3571.
9. „ „ „ 3572.
10. „ „ „ *Cawnpore.*

It may be mentioned here that *Torulopsis utilis* var. *Cawnpore* was subsequently shown to be a variety of *S. cerevisiae* from its ability to form spores and certain morphological features. This observation was corroborated from its chemical nature subsequently by the Bengal Immunity Co., Ltd. But this name is being retained by the present paper as it was supplied under that name.

III. Sporulation Studies

Sporulation is an essential prerequisite in any hybridization programme in the *Saccharomyces* species or the sporulating yeasts. In yeast, sporulation is said to be a form of resistance which allows these organisms to remain viable even though active budding has stopped. It also plays an important rôle in the hibernation of these micro-organisms permitting them to pass over the winter in the ground of vineyards where they are deposited in the autumn, in cold countries. Sporulation is also observed in old cultures where food is scarce, and also in certain solid media such as carrots or gelatin which are not very favourable for budding (Guillermond and Tanner, 1919).

Ascospores were first observed by Schwann (1839) and described by Scynes. It was at that time thought to be a form of encystment resulting from some pathological processes. On the other hand De Bary (1866), Rees (1870) and Hansen (1883) 'likened sporangia of the yeasts to the asci of the Ascomycetes and regarded the yeasts as a group of fungi'. This observation has been later confirmed by Guillermond and Tanner (1919).

According to Guillermond and Tanner (1919) and Hansen the ascospores are often derived from cells which have not ceased to bud. They think that there are no clear cut limits between budding and sporulation, for both are able to be carried on at the same time, in the same cell. Budding continues and slows up only at the time sporulation begins. But the results of the present authors are not at all in conformity with the above observations. Our results with the several species and varieties of *Saccharomyces*, listed earlier, prove that while sporulation and budding may take place in a colony at the same time, in no instance were we able to see a bud on an ascus. This is also in conformity with the physiological conditions of the sporulating and vegetative cells and is substantiated by our staining reactions. We have originated a new differential staining technique by which the vegetative or budding cells can be clearly demarcated from the sporulating cells and by this differential stain we can even distinguish the cells which have stopped their

vegetative activity and may later develop into an ascus. (This technique will be described in a subsequent publication.) Such cells we propose to call ascus mother cells. We thus find three kinds of cells in colonies which are sporulating: (1) the vegetative cells, (2) the ascus mother cell, and (3) the ascus bearing the ascospores, the relative proportions of which will depend on the age of the colony and the conditions of the medium. We also find that in the absence of our differential stain, in certain instances, a small ascospore at the edge of an ascus may give the false appearance of a bud on the ascus. But when such cells are stained, the true nature of the so-called bud is brought out.

Conditions for inducing sporulation

According to Klebs (1900), Guillermond and Tanner (1919) and others, the formation of the ascospores is determined by the lack of food. But yeast also sporulates in certain solid media, like gelatin, slices of carrot and potato, and also at times in liquid media during fermentation. Klebs explains the latter phenomenon also as due to the lack of nutrition when he contends that in solid media, sporulation is limited to cells in the innermost parts of the colonies where they find themselves in bad conditions of food supply. At the same time, the cell on the margin of the colony continue to bud. But Hansen (1902) showed that the cell at the periphery of the colony also sporulate and according to him sporulation is caused by lack of food in certain media, while in others, it is due to the accumulation of the toxic excretions of the cells. Thus with yeasts on gypsum blocks or in distilled water, it is the lack of food that is probably responsible for inducing sporulation, while in solid media like gelatin or carrot slices, it is the action of the toxic excretions which arrests budding and causes sporulation. Sporulation in fermenting solutions can also be attributed to the same phenomenon. It is also known that certain chemical substances, such as calcium sulfate, also induce sporulation (Hansen, 1902).

Saito (1916), on the other hand, opines that the cells on the periphery of a colony sporulate first, but gives the same reason for it—lack of food. But the investigations of Hansen (1902), Barker (1902) and others indicate that apart from the lack of food and accumulation of toxic substances, there are a number of secondary factors, such as free access of air, an optimum temperature and humidity, which control sporulation. It is mainly the researches of Hansen (1902) that have thrown a great deal of light on these secondary factors. He found that in *S. cerevisiae* and *S. Pastorianus*, in the absence of air, no sporulation takes place, although the temperature was kept constant in the treatments. By another series of experiments he showed that it is the oxygen of the air that is responsible for inducing sporulation, and not nitrogen. Regarding the influence of temperature, he showed in six varieties of yeast that the optimum for inducing maximum sporulation is near about 25°C. Hansen also compared the range of temperature for budding and sporulation and found that the minimum temperature for sporulation is not as low as that for budding and maximum is not so high.

Hansen also thinks that a certain amount of humidity is necessary for the induction of ascospores, but Naegeli opines that the principal factor in sporulation is desiccation and that this phenomenon is brought about only in cells which are partly dried. Although the experiments conducted by Hansen seem to be conclusive, our experience is not in conformity with his views. In one set of sporulation studies, we obtained a very high percentage of sporulation, when the cells were inoculated in oversterilized dry wort agar media. But it may be mentioned that in these cases, the relative dryness may not have been solely responsible for sporulation. The factors responsible are under investigation and will be published in due course.

The influence of light of different wavelengths in inducing sporulation was investigated by Purvis and Warwick (1907). They found that the red rays of longer wavelength accelerate the formation of ascospores, the green rays retard ascospore formation to a certain extent, while the blue or violet rays inhibit it to a greater degree. The ultraviolet rays have the most pronounced retarding action, which at the same time affect the vitality of the cells. According to Guillermond and Tanner (1919) these phenomena can be explained on a chemical basis, since the rays of short wavelength have a greater chemical activity than those of long wavelength. Hence the former induce more chemical changes in the cells which are detrimental to sporulation than the latter. But by our staining reaction (mentioned earlier) we find that there is a greater change in the cytoplasm of the ascus mother cells and ascospores than in the vegetative cells. This is not in agreement with Guillermond and Tanner's views.

The researches of Saito (1916) indicate that the *p*H of the medium controls sporulation to a great extent. More work is necessary before this point can be confirmed.

Stantial (1931) showed that several races of *Saccharomyces* sporulated if the cells were agitated at 25°C. in rocker tubes with dilute grape fruit juice. It was found that the percentage of ascus obtained depended upon the number of yeast cells used with a given volume of juice or a given weight of sugar. If the ratio between these quantities is either too great or too small, no asci at all are formed, but the amount of water in the sporulating medium is unimportant. It was also found that sodium or potassium acetate act as sporulation media; but solutions containing both an acetate and a sugar, preferably manose or dextrose, gave the best results. She also thinks that the theory widely held, that well-nourished cells when suddenly brought into unfavourable conditions will form spores, is based on insufficient data.

The methods of inducing sporulation, employed in this Laboratory, are described below. Before attempting sporulation, it is necessary that the cells should be well nourished and young. According to Guillermond and Tanner (1919) they should have acquired sufficient reserve in their protoplasm to assure the formation of ascospores. Thus, according to them, before sporulation, it is necessary to cultivate the yeast in a nutrient medium such as beer wort for about 48 hours with frequent transfers.

(1) *Plaster Block Method.* This has been the standard method since the time of Hansen. The details of this are sufficiently well known and so are not given here. Both distilled water and dilute peptone solutions were used in the experiments, but the percentage of sporulation was very low—about 3% in the *Saccharomyces* species and varieties.

(2) *Modified Gorodkowa's agar* composed of glucose 0·25%, meat extract 1%, NaCl 0·5%. This modification of the original formula of Gorodkowa (1908) has been claimed by many investigators to be an excellent medium for sporulation, but even after a number of repeated trials, we could not get satisfactory results.

(3) The *Cucumber wedge* method described by Mark and McClung (1940) also did not give satisfactory results.

(4) *Potato wedges* and *carrot slices* were also tried, but gave only poor results.

(5) The method described by Stantial (1930) of using *aerated solutions of certain sugars* also did not prove satisfactory.

(6) Cane sugar solution 10% or glucose 4%. These gave about 10–20% sporulation, depending upon the varieties used.

(7) *Oversterilized and dry wort agar* media gave the best results. This method with certain modifications was used in the present studies and gave about 35–45% sporulation in the following types:—

1. *S. cerevisiae* var. B.I.
2. „ „ 2160.
3. „ „ 918.
4. *T. utilis* var. *Cawnpore.* (This, as stated above, appears to be a variety of *S. cerevisiae.*)

IV. Hybridization

The technique employed in the present experiments is a modification of the methods used by Winge and Laustsen (1938) and is described elsewhere in the paper. The main object of the present work is to demonstrate the possibility of producing yeast hybrids of commercial importance and to elucidate the value of taxonomy of the *Saccharomyces* and *Torulopsis.*

Winge and Laustsen (1939) proceeded with their breeding experiments in the following manner. Single spores were isolated from several strains of *S. cerevisiae*, *S. Italicus*, *S. validus*, *S. Mandschuricus* and *Zygosaccharomyces Priorianus.* These spores were brought together in pairs when conjugation was observed to take place, subcultures were made with various characters, particularly giant colonies were observed. The giant colonies of 14 new hybrids differed in contour and texture, from both the parent types. The hybrids were also compared with the parental types as regards their ability to ferment sugars. In all cases, ability to ferment was a dominant character, for instance, if one of the parent types fermented sucrose and the other did not, the hybrid invariably fermented sucrose. It may be mentioned that a similar character developed in two of the hybrids made at this Laboratory which is being dealt with elsewhere in this paper.

The operating chamber used in the experiment was made at the workshop of the Bose Research Institute and is a modified form of the chamber devised by Winge and Laustsen (1938). It consists of a glass ring about 2 cms. in diameter and 1 cm. high into which two oval glass tubes, of lumen 1·5 cms. × 0·8 cm. and about 2 cms. in length, are inserted at both the sides. This chamber is fixed to a thin microscopic slide by means of a mixture of paraffin and vaseline. The chamber and the slide are sterilized before the operations. This is then placed on the platform of a microscope in such a way that the two needles of the micromanipulator pass through the two arms of the operating chambers. The entrance is closed with moist cotton during the operation and plugged with the same after the operation.

The micromanipulator used in the experiments is of the Zeiss type with one micro-movement arm on each side. The glass micro-needles used in the experiments were made at the workshop of the Bose Research Institute. They consist of finely drawn out glass rods bent at the tip, at about an angle of 45°. Two kinds of needles were made, one having the point drawn out to about 5 μ and the other to about 1 μ. One of each kind was fitted to the arms of the micromanipulator.

For the cultivation and preliminary examination of the asci, ordinary glass rings, finely ground on both sides about 2 cms. in diameter and 1 cm. high, were used. This was fixed to a thin slide using a mixture of paraffin and vaseline. The chamber was kept moist by adding one or two drops of distilled water after sterilizing the chamber. Small samples of yeast consisting of a group of cells were taken on a cover slip in a hanging drop of glucose-peptone broth and if there are a sufficient number of asci in the sample, this

cover slip was transferred to the operating chamber described earlier. Then a single ascus was carefully removed with the micro-needle and placed in a hanging drop of glucose-peptone on another cover glass. Similarly an ascus of the other parent was also removed to another hanging drop on the same cover glass and another empty drop of glucose-peptone broth was also placed on the same cover glass in a convenient place. Now the first ascus was crushed with the thicker needle by pressing the ascus between the tip of the upturned micro-needle and cover glass, by using the minimum pressure necessary for the operation. The operation at the same time was observed through the microscope. When the ascospores came out, one of them was carefully removed by the tip of the thinner needle to the empty drop, on the cover slip.

The ascus of the other parent in the same cover glass was now crushed in the same manner, and one of the spores removed in the same way and placed as near as possible to the previous ascospore in the new drop. Then the chamber was sealed with moist cotton wool and the spores were left near each other to fuse. The spores were constantly observed to note the time taken for fusion. In favourable types, it was observed that they fused within 8 to 12 hours. In a few instances, the ascospores started budding without fusion, which may give to a haploid colony. Sometimes any two of the cells thus formed may fuse later to form a diploid cell, as was observed by Winge and Laustsen (1938). The operation described above is of a very delicate nature and considerable skill in the manipulation should be acquired before satisfactory results could be obtained.

The following were the crosses attempted:—

	Parents		Name of the cross
*1.	*S. cerevisiae* var. B.I.	× *S. cerevisiae* var. 2160	S.C.H. 75
*2.	„ „ „	× „ „ 918	S.C.A.
*3.	„ „ „	× *T. utilis* „ *Cawnpore*	S.C.B.T.
4.	„ „ 2160	× *S. cerevisiae* „ 918	
5.	„ „ „	× *T. utilis* „ *Cawnpore*	
*6.	„ „ 918	× „ „ „	T.S.
*7.	S.C.H. 75	× *S. cerevisiae* „ B.I.	S.C.H. 76
8.	„ 75	× „ „ 2160	
9.	„ 75	× *T. utilis* „ *Cawnpore*	
10.	„ 75	× *S. cerevisiae* „ 918	
11.	S.C.A.	× *S. cerevisiae* „ B.T.	
12.	„	× „ „ 918	
13.	„	× „ „ 2160	
14.	„	× *T. utilis* „ *Cawnpore*	
15.	T.S.	× „ „ „	
16.	„	× *S. cerevisiae* „ 918	

The following were the attempts at intergeneric hybridization. Here, excepting in *T. utilis* var. *major* Y41, a single spore of the *S. cerevisiae* variety was placed near a single cell of the *T. utilis* varieties, thus bringing together the two haploid elements. But in the case of *T. utilis* var. *major* Y41, a single vegetative cell was placed close to another vegetative cell of the *S. cerevisiae* varieties, in which case both the cells are supposed to be diploids and the colony resulting from the fusion, if any, will be tetraploid.

* Denotes cases of successful crossing.

17.	*T. utilis* var.	*major*	Y41	×	*S. cerevisiae* var.	B.I.
18.	„	„	„	×	„	„	2160
19.	„	„	„	×	„	„	918
20.	„	„	„	×	S.C.H. 75		
21.	„	„	„	×	*T. utilis* var.	*Cawnpore*	
22.	„	„	3571	×	*S. cerevisiae* var.	B.I.	
23.	„	„	3571	×	„	„	2160
24.	„	„	„	×	„	„	918
25.	„	„	„	×	S.C.H. 75		
26.	„	„	„	×	S.C.B.T.		
27.	„	„	„	×	S.C.A.		
28.	„	„	„	×	*T. utilis* var.	*Cawnpore*	
29.	„	„	3572	×	*S. cerevisiae* var.	B.I.	
30.	„	„	„	×	„	„	2160
31.	„	„	„	×	„	„	918
32.	„	„	„	×	S.C.H. 75		
33.	„	„	„	×	S.C.B.T.		
34.	„	„	„	×	S.C.A.		

It will be seen that out of a total of 34 combinations, only 6 proved to be successful, including a single case of intergeneric cross. It may be mentioned that in all the above cases, the actual fusion was observed to take place. But this high percentage of failure may be due to any one of the following causes:—

(1) The ascospores or the cells may have been injured during the operation. This cannot always be prevented as the operation is very delicate and rather difficult. To rule out this possibility, the same combinations were tried a number of times, but with the same negative results.

(2) The incompatibility of the parents due to differences in the chromosome or genetic constitution. This can be established only after ascertaining that the separated ascospores were not injured in any way during the process of micromanipulation. This is not possible under the microscope as the spores are very small, about $\frac{1}{2}$ to 1 μ in diameter. But it is hoped that a cytogenetical study of the parents may throw some light on this problem, and this work has already been undertaken.

From the above table, crosses 1, 2, 6 and 17 were supplied to the Bengal Immunity Co., Ltd., for testing the yield and the biochemical properties and at the time of writing this paper, the full data have not been received by us. But it may be mentioned that in cross No. 1 (S.C.H. 75) it was reported that while the parents gave an yield of about 12-13%, the hybrid gave about 19%, showing an increase of about 7%. Moreover the cells of this hybrid were bigger than either of the parents.

The importance of hybridization in producing new ultimate characters may be seen from the reported chemical behaviour of the following cross. While *S. cerevisiae* var. 918 does not utilize either nitrates or alcohol and *S. cerevisiae* var. B.I. utilizes only nitrates, the hybrid between the two (S.C.A.) was found to utilize both nitrates and alcohol. Again, the hybrid between *S. cerevisiae* var. 918 and *T. utilis* var. *Cawnpore* was also observed to utilize both nitrates and alcohol when the former parent does not utilize either, while the latter utilizes only nitrates but not alcohol.

The generation time of the parents and the hybrids was next considered. A correct idea of the generation time is essential in the breeding programme. In cases where the generation time of a hybrid is greater than any one of the parents, the hybrid has to be repeatedly back-crossed with the parent having the lower generation time. This may tend to increase the yield of the hybrid.

The following table gives the generation of some of the types investigated:—

Name of yeast	Time taken for the first bud to appear from a single vegetative cell	Time taken by the first bud to develop to its normal size	Time taken by the second bud to develop to its normal size	Time taken by the third bud to develop to its normal size	Time taken by the fourth bud to develop to its normal size
S. cerevisiae var. B.I. ..	155 mts.	95 mts.	71 mts.	65 mts.	65 mts.
„ „ 2160 ..	150 „	110 „	75 „	55 „	55 „
„ „ 918 ..	170 „	130 „	80 „	60 „	60 „
S. ellipsoideus var. 4097 ..	120 „	75 „	60 „	55 „	55 „
„ „ 4108 ..	135 „	99 „	75 „	65 „	65 „
T. utilis var. *major*	170 „	120 „	90 „	55 „	55 „
„ „ 3571	150 „	80 „	70 „	65 „	65 „
„ „ 3572	110 „	90 „	85 „	60 „	60 „
„ „ *Cawnpore* ..	140 „	109 „	90 „	55 „	55 „
S.C.H. 75	133 „	100 „	75 „	70 „	70 „
S.C.A.	110 „	80 „	60 „	55 „	55 „
T.S.	120 „	90 „	75 „	65 „	65 „

In conclusion, it may be mentioned that irradiation experiments are being conducted on the yeast species and varieties and significant results have already been obtained. These will be described in a subsequent paper. We also undertook a critical detailed study of the vacuoles, as an aid to the classification, by using vital stains such as Janus green, Methylene blue and Neutral red. It was found that certain regular patterns could be ascribed to the various species and varieties which will also be dealt with in a subsequent paper. These vacuolar studies have tended to clear up certain misconceptions in the classification of the yeast and we also propose to classify the yeast on the above basis.

V. Acknowledgments

It is with great pleasure that we acknowledge the award of a generous grant by the Bengal Immunity Co., Ltd., for carrying on the above investigations at the Bose Research Institute. Our thanks are also due to the Director of the Bose Research Institute for giving us every facility for carrying out these investigations and the keen interest he has taken in the progress of the work.

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XI. PRODUCTION OF PENETRATING MULTIPLES

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INTRODUCTION

The existence of penetrating multiples which do not produce secondaries like the soft component has been proved by the investigations of several workers. Schein, Jesse and Wollan,[1] Carlson and Schein[2] have obtained evidence of their existence in upper atmosphere. Swan and Ramsay,[3] D. S. Santos, Pompei, Wataghin,[4] Janossy[5] have also confirmed their existence at sea-level by different counter arrangements. Powell,[6] Rochester, Janossy and McCusker,[7] Sinha and Sengupta[8] have obtained Wilson Chamber photographs of showers containing penetrating particles recognized by their non-production of secondaries in lead.

The experiments of Janossy are by far the most extensive in this direction. But his experiments suffer from the inherent uncertainty of counter experiments as regards the nature and number of particles constituting a shower. Moreover the penetrating showers investigated by Janossy are very hard which penetrate more than 50 cm. of lead. There is also another defect in the experiments of Janossy due to the fact that there are gaps in his central counter system H (cf. p. 362 of ref. 5). His results show that the maximum number of records is obtained for $n = 0$, when the particle or particles passing through the upper and lower system of counters have not excited any of the middle counters 'H' and have passed through the gaps between them. Janossy's data are more or less concerned with very hard penetrating multiples already present in the atmosphere; he finds that almost all of them are associated with extensive air showers. He has nevertheless studied the transition effect of these showers in lead and aluminium and found a Z^2 dependence. But he has always placed 30 cm. of lead in the path of the rays, so that slow penetrating particles have not been recorded at all.

The present investigation, on the other hand, deals with the production of penetrating multiples and their transition effect in small thicknesses of lead where practically no experimental data are available. This region is theoretically the most significant as will be seen later from theoretical discussions.

GENERAL EXPERIMENTAL RESULTS

The counter arrangement used in the experiment was a five-fold coincidence one and is shown in Fig. 1. The arrangement is such that any shower which contains at least two particles within an angle θ ($9{\cdot}5° > \theta > 2{\cdot}5°$), subtended by the tangents to the lowermost counters at the bottom of the top absorber, would be recorded. A preliminary qualitative analysis of the experimental results on the penetrating showers has appeared before. The penetrating character of the multiples was ascertained from their non-production of secondaries in the 2·2 cm. lead plate inside the chamber. The main result was that the rate of penetrating multiples as a function of the thickness of lead placed above the top-counter gave two maxima at 5 cm. and 15 cm., the former being steeper than the latter showing thereby that the mesons produced in 5 cm. of lead contain a larger

fraction of low energy mesons than those produced in 15 cm. Though the rate of shower decreases after 5 cm. and then increases at 15 cm. the density of meson-showers, i.e. the average number of particles per shower, increases monotonously with the lead thickness. Qualitatively we therefore arrive at the conclusion that with the increase in energy of the primary component responsible for the production of secondary meson-multiples, both the average number of particles per shower as also the average energy per particle in-

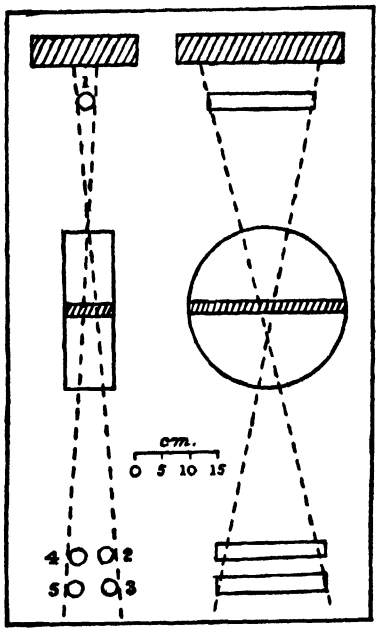

FIG. 1. Counter Arrangement.

crease. This is in qualitative agreement with the theory of multiple production by Euler and Heisenberg,[10] who give the relation

$$\bar{\epsilon} = \mu ln \frac{\epsilon}{\mu}; \quad \bar{n} = \frac{\epsilon}{\mu ln \frac{\epsilon}{\mu}}$$

where $\bar{\epsilon}$ and $\bar{n}$ are the average energy and number of mesons produced by a primary radiation of energy ϵ and μ the rest energy of a meson. The monotonous increase of the multiplicity (i.e. the size of the showers) with lead thickness also agrees qualitatively with the recent theory of Heitler and Peng,[11] in which they find that the probability of production of a multiple of n particles from a primary meson has separate maxima for different values of n (1, 2, 3, etc.) and the position of this maximum shifts to higher energies for higher values of n.

We shall in this paper try to find out the cross-section for the process of formation of meson pairs from primary mesons by scattering in the nuclear field of protons and neutrons. Before doing this we shall give a general summary of the existing theories for the production of meson-multiples.

THEORETICAL

There are mainly two theories regarding the process of creation of meson-multiples. The first is due to Heisenberg [12] which has been modified by Oppenheimer and Schwinger [13]

for scalar and pseudoscalar mesons. The second which is more recent and more exhaustive is due to Heitler and Peng.[14] We shall consider these two theories in brief.

(i) *Heisenberg*

In both the theories it is generally assumed that when a fast proton (or neutron) is scattered by another nucleon at rest a part of the proper meson field associated with the colliding proton is radiated away as a wave-packet, whose energy for a short collision time is equal to the difference in the proton field before and after the collision. The meson field is given by a scalar charge g, and a vector moment f, such that the interaction between the meson field and the nucleon is proportional to

$$\gamma = \frac{g^2}{\hbar c} \quad \text{and} \quad \Gamma = \frac{f^2}{\hbar c}.$$

The meson field associated with the colliding nucleon in Heisenberg's model is represented by a wave-packet with total energy

$$\epsilon = \int f(k_0)dk_0 \quad .. \quad .. \quad .. \quad .. \quad .. \quad (1)$$

where k_0 is the momentum given by $k_0 = p/h$. Now if $\epsilon > \chi$, χ being the meson mass $\left(\chi = \frac{\mu c}{\hbar}\right)$, if c and $\hbar$ are taken as unity, then the wave-packet will lead to the creation of one or more mesons each of energy

$$\epsilon^2 = \chi^2 + k_0^2.$$

If $\alpha(k_0)dk_0$ is the probability of a meson occurring in the interval dk_0, then

$$\alpha(k_0) = \frac{f(k_0)}{k_0}$$

and the average number of mesons emitted is given by

$$\bar{n} = \int_{\chi}^{\epsilon} \frac{f(k_0)dk_0}{k_0} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (2)$$

The problem is to find out the nature of the distribution function (1) for the wave-packet. For a charged scalar field the distribution is found to be

$$f(k_0)dk_0 = \text{constant}.$$

This leads to $\bar{n} = \text{constant}. \; ln \frac{k_{max}}{\chi}$

where k_{max} is the upper energy limit which is determined by the time of collision. This formula is very similar to that given by Oppenheimer[13] *et al.* for a scalar meson field. The average number in the latter case is

$$N \sim \frac{1}{3\pi} \gamma ln \left(\frac{10\epsilon}{\chi}\right).$$

For the vector meson field $g = 0$ and $f \neq 0$. The distribution function (1) for this case as proposed by Euler, which according to him gives the best agreement with experimental results, is

$$f(k_0)dk_0 = \frac{a}{k_0} dk_0$$

where a is the spatial extension of the source and

$$\epsilon = \int_{\chi}^{\epsilon} f(k_0)dk_0 = a\int_{\chi}^{\epsilon}\frac{dk_0}{k_0} = a\ln\frac{\epsilon}{\chi} \quad .. \quad .. \quad .. \quad (3)$$

$$\bar{n} = \int_{\nu}^{\epsilon}\frac{f(k_0)dk_0}{k_0} = a\int_{\nu}^{\epsilon}\frac{dk_0}{k_0^2} = a\left(\frac{1}{\chi}-\frac{1}{\epsilon}\right) = \frac{a}{\chi}$$

where ϵ is very large and $\frac{1}{\epsilon}$ can be neglected.

$$\chi\ln\frac{\epsilon}{\chi}$$

substituting for a from (3), and the average energy

$$\bar{\epsilon} = \frac{\epsilon}{n} = \chi\ln\frac{\epsilon}{\chi}.$$

These equations for $\bar{n}$ and $\bar{\epsilon}$ have already been found to be in qualitative agreement with our experimental results.

It has been shown by Heisenberg that provided the energy contained in the wave-packet is large compared to the meson rest energy, the packet can give rise to either 1, 2, ... or n_{max} mesons, the probability of whose occurrence will be given by Poisson's fluctuation formula,

$$\frac{e^{-\bar{n}}.n^{\bar{n}}}{n!}$$

where n is the mean number of mesons.

(ii) *Heitler and Peng* [14]

The theory of meson production due to these authors is the meson analogue of the production of bremhstrahlung when a fast charged particle is deflected in the coulomb field of another charged particle. The processes possible are

$$\left.\begin{array}{l} P+P \rightarrow P+N+M^+ \\ P+N \rightarrow P+P+M^- \\ N+N \rightarrow N+P+M^- \\ N+P \rightarrow N+N+M^+ \end{array}\right\} \quad .. \quad .. \quad .. \quad .. \quad (4)$$

The meson field of a fast nucleon is virtually equivalent to a superposition of plane waves representing a beam of free mesons of various energies, provided that the velocity of the incident nucleon is almost equal to c, i.e. its energy E is large compared to its rest energy Mc^2. The virtual meson field of the fast nucleon has a certain energy distribution $q(\epsilon)d\epsilon$, which has been derived by Heitler and Peng.[14] Each of these virtual mesons will be scattered by the nucleon at rest. Let us suppose that one of the scattered mesons has an energy ϵ' and $Q(\epsilon, \epsilon')d\epsilon'$ be the cross-section for scattering. Then the cross-section for the production of a meson of energy ϵ' in a collision of two nucleons will be given by

$$\phi d\epsilon' = d\epsilon' \int Q(\epsilon, \epsilon')q(\epsilon)d\epsilon \quad .. \quad .. \quad .. \quad .. \quad (5)$$

where the integration is to be carried over all virtual mesons. The validity of this method is, however, limited by the condition

$$E \gg \epsilon \gg \mu c^2.$$

The scattering cross-section used in the above equation has been calculated by taking proper account of radiation damping. Further in carrying out this integration, two things have to be kept in mind.

(i) The energy ϵ of the *equivalent* meson is the energy lost by the moving nucleon and equal to the sum of the energy ϵ' of the meson produced and the energy transferred to the stationary nucleon. In non-relativistic case the latter is negligible.

(ii) Equivalent mesons of different polarization may contribute to the production of a meson of particular polarization, restricted, however, by some selection rules. The coupling constants for transverse, pseudoscalar and longitudinal mesons are given by f^2, f'^2 and g^2 respectively where

$$f^2 = f'^2 = 0{\cdot}13 \text{ and } g^2 = 0{\cdot}054.$$

It is the fast moving nucleon that loses the charge, since the creation of a meson is due to the scattering of one of the equivalent mesons of the colliding nucleon by the scattering nucleon (stationary) which does not change its charge. The selection rule referred to in (ii), as found by Heitler and Peng, is that a pseudoscalar meson is almost always scattered into a longitudinal meson. The occurrence of these selection rules is entirely an effect of damping and is one of the most characteristic features of Heitler and Peng's theory.

The largest contribution to the energy spectrum $q(\epsilon)d\epsilon$ comes from the equivalent transverse mesons, and next from pseudoscalar mesons, but very little from the longitudinal mesons. The total cross-section for the production of only transverse mesons (from which the largest contribution comes) as calculated from (5) is very large and is given by

$$\phi = 3{\cdot}0 \times 10^{-25} \text{ cm.}^2\text{/per nucleon} \quad \ldots \quad (6)$$

In the process of collision the moving nucleon loses an energy ϵ which is divided between the meson produced which gets an energy ϵ' and the recoil nucleon which gets an energy $\epsilon - \epsilon'$. The recoil nucleon is further capable of producing mesons and recoil nucleons by collision with other stationary nucleons. This process will be repeated until the kinetic energy of the recoil nucleon falls to a value equal to its rest energy Mc^2 when the probability of its creating fresh mesons will be very small. The process is almost like the cascade multiplication of soft particles bremhstrahlung generation, but is not easily observed since the energy distribution of the recoil nucleons in the above case favours low energies more strongly than in the case of bremhstrahlung emitted by a soft particle.

The fast moving nucleon with energy $E - \epsilon$ can again collide with another nuclear particle at rest and can produce another meson. Though the above process envisages a cascade production of mesons, it has been shown by Janossy[14] that the cross-section given in (6) is so large that a heavy particle crossing an atomic nucleus is expected to collide several times within the same nucleus and hence meson showers observed in cloud chambers appearing to diverge from a point can be due to a process described above instead of being produced in one single act.

There is one more process by which a fast heavy particle colliding with another at rest can create mesons. The field of a fast nucleon, when expanded into a Fourier series, is equivalent (i) to a beam of light quanta arising from the coulomb field as well as (ii) to a beam of virtual mesons arising from the nuclear field. The second process will give rise

to mesons by scattering in a way described above whereas the light quanta can produce mesons by the process

$$\hbar\nu + P \rightarrow N + M^+$$
$$\hbar\nu + N \rightarrow P + M^-.$$

It has been shown by Hamilton and Peng [15] that the contribution of (i) is entirely negligible compared to that of (ii) except when the energy of the moving nucleon is greater than 10^{12} e.v.

In all the calculations given by Hamilton, Heitler and Peng [16] the value of the cross-section for scattering $Q(\epsilon, \epsilon')d\epsilon'$ used is its asymptotic value for higher energies. Further it has been shown by Heitler and Peng [11] that when a fast meson is scattered by a nucleon, the meson instead of being scattered as a single meson, can split up into two or more mesons. This multiple creation has been entirely neglected above. We shall now briefly consider this process which is the most important for our purpose.

(iii) *Meson-Nucleon Collision*

The problem of a meson-nucleon collision is primarily the problem of the scattering of the meson in the nuclear field of a proton or neutron. When a fast meson collides with a nucleon at rest and the method of expansion according to the interaction parameter 'g' (corresponding to the charge e in the radiation theory) is applied, it is found that above a certain energy the meson can not only be scattered by the nucleon, but also can split up into a number of secondary mesons according to the processes

$$M^+ + N \rightarrow P + M^+ + M^-$$
$$M^- + P \rightarrow N + M^+ + M^-.$$

The cross-section for the processes increases the more rapidly with energy the higher the multiplicity of the process is. This is, however, clearly impossible and it shows that the method of expansion completely fails at higher energies. It has been shown by Heitler and Peng [17] that after the diverging integrals have been omitted, a set of equations can be obtained which is free from any singularities and differs from what is obtained by the usual expansion method by the inclusion of radiation damping. More recently Peng [17] has found that the provisional method of including radiation damping followed by Heitler and Peng [11] can be rigorously established.

As a first approximation, the production of three or more mesons has been neglected. The result will be quite valid for the energy range $10^8 \rightarrow 6\times10^8$ e.v. The cross-section for the splitting up of a meson into two is then given by

$$\gamma_2 = \frac{4}{5} g^6 \epsilon^4 \text{ for}$$

and

$$\gamma_2 = \frac{144\pi^2}{\sigma^6 \epsilon^8} \text{ for } g\epsilon \gg 1$$

where the cross-section is expressed in units of $(\hbar/\mu c)^2$, i.e. $4{\cdot}3\times10^{-26}$, ϵ is the energy of the incident meson in units of μc^2 and g is the interaction parameter for a longitudinal meson of spin 1.

The curves for both γ_1 (which is the cross-section for the first-order process of scattering into a single meson) and γ_2 as functions of the energy of the incident mesons are reproduced in Fig. 2. The dotted portion is not very accurate, for in this energy region the theory is no longer valid as processes of higher multiplicity come in. But it will not

be incorrect if we say that the cross-section decreases very rapidly with energy after 10^9 e.v.

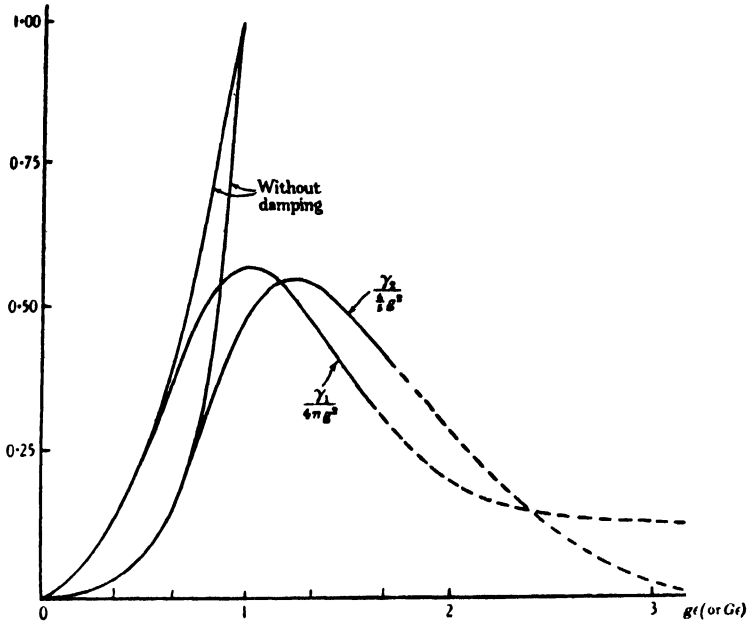

FIG. 2. γ_1 and γ_2 are the cross-sections for the scattering of a meson by a neutron and the double process $Y^+ + N = P + Y^+ + Y^-$. Both are in units of $(\hbar/\mu c)^2$. The dotted parts are unreliable owing to the influence of processes of higher multiplicity. The curves that would be obtained if damping were neglected are also given. g is approximately $\frac{1}{3}$. (For Ge see §V at the end.)

For the higher multiplicities, it has been found that if γ_n represents the cross-section for the nth multiplicity it is found that γ_n has a maximum (similar to γ_2) which shifts to higher energy values for higher values of n.

COMPARISON WITH EXPERIMENT

It has already been shown that the cross-section for the production of mesons from a nucleon-nucleon collision is so high that a fast nucleon is capable of colliding several times within a single atomic nucleus. This process of meson production is important only in high altitudes, for very few fast protons or neutrons will reach sea-level without multiplying into mesons long ago. The process of a photon multiplying into a meson pair is taken to be negligible at ordinary energies (less than 10^{10} e.v.). In fact, the theoretical cross-section for the materialization of a photon into a meson pair could account for only 0·4% of the multiples found out by Janossy [5] experimentally. So we shall assume that the two-meson multiples that are produced in the first 5 cm. of lead at sea-level are all due to splitting up of a meson.

In order to compare our experimental results with the theory of Heitler and Peng,[11] we have to integrate the cross-section over the whole energy spectrum of mesons, since mesons of all possible energies were incident on the lead absorber placed above the cloud chamber. Of course, there will be very little contribution from mesons of energy greater than 10^9 e.v. (see Table II).

If the number of mesons incident on the top absorber in the energy interval E and E+dE is

$$dn = N(E)dE$$

then the total cross-section for the production of meson pairs from mesons of this energy interval is

$$P(E) = \gamma_2(E)N(E)dE$$

where $\gamma_2(E)$ is the value of the cross-section for the energy range between E and E+dE. Therefore the average cross-section for the whole energy spectrum would be

$$\frac{\int_0 \gamma_2(E)N(E)dE}{\int_0^\infty N(E)dE} \quad .. \quad .. \quad .. \quad .. \quad (7)$$

The energy spectrum of mesons has been accurately determined by Hughes[18] for mesons of energy greater than 5×10^8 e.v. and the spectrum below this energy has been roughly obtained by Williams[19] from tracks photographed in random expansions of a cloud chamber. Blackett[20] first gave the energy spectrum of all cosmic ray particles in which both electrons and mesons were included. After a careful study of all these investigations Table I has been computed. This table gives the number of mesons of each energy interval expressed as percentage S of the whole energy spectrum. That is, it gives the value of S, where

$$S = \frac{N(E)dE}{\int_0^\infty N(E)dE} . 100.$$

The right hand expression of equation (7) has been calculated graphically taking the energy spectrum to be given by Table I. In making this calculation it is to be remembered that the energy as given in Table I is actually the momentum pc, for it is determined from the curvature of the tracks in a magnetic field. The true energy will be given by

$$E^2 = p^2c^2+(\mu c^2)^2.$$

The correction due to this will be less than 1% after 6×10^8 e.v., but about 40% in the energy range 1 to 2×10^8 e.v. For example, the range $1\rightarrow2\times10^8$ in Table I corresponds to an energy range $1{\cdot}41\rightarrow2{\cdot}2\times10^8$ e.v.; similarly $2\rightarrow4\times10^8$ corresponds to $2{\cdot}2\rightarrow4{\cdot}1\times10^8$ e.v.; and $4\rightarrow6\times10^8$ corresponds to $4{\cdot}1\rightarrow6{\cdot}0\times10^8$ e.v. The values of $pc>6\times10^8$ can be taken to be equal to the energy without any appreciable error.

TABLE I

Energy spectrum of mesons

	10^8 e.v. region					10^9 e.v. region					
pc	1→2	2→4	4—6	6—8	8—10	1—2	2—4	4—6	6—8	8—10	$10^{10}\rightarrow\infty$
S	2·2	4·6	5	5·5	6·2	28·5	20	6·6	2·4	1·5	13

4·5% of $pc<10^8$ e.v.

In carrying out the calculation for the theoretical value of σ for the whole energy spectrum, the cross-section γ_2 were noted down at intervals of 2×10^8 e.v. from Fig. 2 and the arithmetic mean of the values of γ_2 at the two limits was then multiplied by S, the percentage of mesons actually present in the particular energy interval as given by Table I. As the corresponding calculation for the nuclear scattering cross-section γ_1 will also be required for a comparison of our results with those of other investigations we have tabulated the values of γ_1 and γ_2 and also the products $S\gamma_1$ and $S\gamma_2$ in Table II. The last column gives the values of $\Sigma S\gamma_1$ and $\Sigma S\gamma_2$. Since S has been expressed as percentage of the whole spectrum, the average cross-section for the whole energy spectrum will be evidently given by

$$\sigma_1 = \frac{\Sigma S\gamma_1 \times 6\times10^{-26}}{100}; \quad \sigma_2 = \frac{\Sigma S\gamma_2 \times 3\cdot8\times10^{-27}}{100}.$$

TABLE II

Integrated theoretical cross-sections σ_1 and σ_2 for the whole energy spectrum of mesons

	10^8 e.v. region					10^9 e.v. region						Integrated cross-section
	1·41 → 2·2	2·2 → 4·12	4·12 → 6	6 → 8	8 → 10	1 → 2	2 → 4	4 → 6	6 → 8	8 → 10	$10^{10}\to\infty$	
γ_1	0·305	0·51	0·324	0·166	0·13	0·055	0·014	0·005	0·0026	0·0016	0·0013	In units of 6×10^{-26} $\Sigma S\gamma_1 = 8\cdot26$
$S\gamma_1$	0·671	2·346	1·62	0·913	0·806	1·57	0·28	0·033	0·0052	0·0024	0·0169	
γ_2	0·10	0·35	0·42	0·18	0·035	Negligible.						In units of $3\cdot8\times10^{-27}$ $\Sigma S\gamma_2 = 5\cdot1$
$S\gamma_2$	0·22	1·614	2·10	0·95	0·216							

The units for γ_1 and γ_2 are 6×10^{-26} cm.^{2} and $3\cdot8\times10^{-27}$ respectively in the above table, for the ordinates in Fig. 2 for γ_1 and γ_2 are actually $\gamma_1/4\pi g^2$ and $\gamma_2/\frac{1}{5}g^2$. For pseudo-scalar mesons the constant g^2 is to be replaced by f^2, which is almost equal to 1/9. The product $S\gamma_2$ was plotted against energy and it was found that it has a maximum at $4\cdot4\times10^8$ e.v. whereas γ_2 individually (in Fig. 2) has a maximum at $3\cdot6\times10^8$ e.v. This shift of the maximum towards higher energy is entirely an effect of the energy spectrum which has a very sharp maximum at $1\cdot2\times10^9$ e.v. The values of σ_1 and σ_2 then come to

$$\sigma_1 = 4\cdot95\times10^{-27} \text{ cm.}^2\text{/per nucleon}$$
$$\sigma_2 = 1\cdot94\times10^{-28} \text{ cm.}^2\text{/per nucleon.}$$

We proceed to find the average cross-section from our experimental data. It will be seen from Table II of reference 9 that during a total time of 151 hours altogether seven two-meson multiples were produced in the 5 cm. of lead above the chamber. In order to calculate the experimental value of σ_2 we have also to find out the number of single mesons that could excite our counter system, i.e. coincidence (123) and coincidence (145) in Fig. 1. Any meson exciting either (123) or (145) would by producing a meson pair excite the five-fold system (12345). To find out this, the triple coincidences (123) and (145) were observed separately for each thickness of lead. The average of these two has been plotted

in Fig. 3. It is found that the single intensity decreases rapidly up to 5 cm. of lead and after 15 cm. the absorption becomes slow and almost linear indicating that only mesons

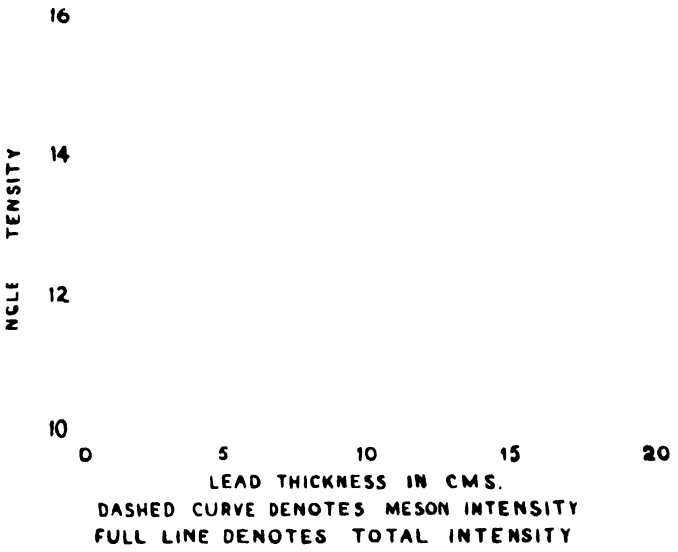

FIG. 3.

are now being recorded. A tangent is drawn to this curve at 15 and produced backward. This would give correctly the meson rate at smaller thickness of lead. The value of the ordinate cut off by this tangent at 5 cm. is 12·6 per hour. Since mesons passing through either (123) or (145) could be effective in producing a two-meson multiple so as to make a five-fold coincidence possible, we have to take double the extrapolated three-fold coincidence rate 12·6 at 5 cm., as the number of mesons that could have possibly produced a meson pair recordable by our counter arrangement. This gives 25·2 mesons per hour or 3805 mesons in 151 hours.

Now if Δ be the fraction of a meson that has produced a two-meson multiple, then the average cross-section per nucleon is

$$\sigma_{2\,\mathrm{exp}} = \frac{\Delta}{tN}$$

where t is the thickness of the material placed in the path of the mesons and N the number of nucleons per cm.^{3} of the material. Inserting values we get

A. $$\sigma_{2\,\mathrm{exp}} = \frac{7}{3805} \times \frac{1}{5 \times 6{\cdot}8 \times 10^{24}} \quad \text{where } \Delta = \frac{7}{3805}\cdot$$

$$= 5{\cdot}4 \times 10^{-29} \pm 25\% \text{ cm.}^2\text{/per nucleon.}$$

Considering the theoretical uncertainties at large energies and also the uncertainties of the coupling constant, we can say that the agreement between the theoretical and experimental values is not bad. The experimental value is lower than the theoretical value by a factor 4. There are two sources of error in this computation but fortunately they are in opposite directions and almost cancel each other.

(i) Two-meson multiples having an angular separation of more than 10° at right angles to the plane of the chamber would not be recorded by our counter arrangement.

These would be very few for the meson multiples generally come very close together. Inclusion of these would make the numerator of Δ larger.

(ii) In our computation of the total number of single mesons responsible for the production of two-meson multiples, we have not taken into account those mesons which excite the top counter (1) in Fig. 1 but pass through the gap between the counters (23) or (45). The area covered by this gap is less than 15% of the area covered by the counters (23) and (45). Inclusion of these mesons would have increased the denominator of Δ.

Thus the two effects tend to counteract each other and hence the experimental value found out above will not be far from true. An independent check of our experimental value deduced from results of other investigations is given below. There are at present no other experimental data for the determination of σ_2; but an indirect comparison can be made from the experimental value of σ_1.

Shutt [21] has found out experimentally the average cross-section for nuclear scattering, i.e. σ_1 for the whole energy spectrum of mesons, and finds the value

$$(a) \qquad \sigma_1 = 6{\cdot}55\times10^{-28} \text{ cm.}^2\text{/per nucleon} \pm 10\%.$$

In order to compare this value with the theoretical value of Heitler and Peng [11] we have already found out the theoretical value of σ_1 for the whole energy spectrum exactly in the same way as it has been done for σ_2. The theoretical value of σ_1 is $4{\cdot}95\times10^{-27}$ which is about seven times larger than the experimental value. Thus the discrepancy between the theoretical and experimental values in σ_1 is in the same direction as has been found out in this experiment for σ_2 and it is also notable that the amount of discrepancy is of the same order of magnitude. In a very recent paper Shutt [22] has recalculated the value of σ_1 and finds the value

$$(a') \qquad \sigma_1 = 1{\cdot}4\times10^{-27} \text{ cm.}^2\text{/per nucleon}$$

for the whole energy spectrum of mesons. If we accept this value the ratio between theoretical and experimental values of σ_1 becomes almost exactly what we have found out for the theoretical and experimental values of σ_2. This is a clear indication that the experimental values discussed here are consistent, and any attempt at modification in the theory to bring down the value of σ_1 would also bring down the value of σ_2 towards the experimental value.

There is one more point to be discussed. The values of σ_1 and σ_2 calculated above theoretically from Heitler and Peng's curves refer to pseudoscalar mesons of spin zero only. If we assume that mesons of all kinds of polarization are produced in the process of scattering, the theoretical values of σ_1 and σ_2 would be increased by a factor 2·5 and thus would be in worse agreement with both Shutt's and the present experiment. This is not in accord with the way Heitler and Peng [23] have compared their results with the experimental values of Shutt. If we assume that mesons of all polarization are produced by scattering, the maxima for γ_1 and γ_2 occur at $1{\cdot}81\times10^8$ e.v. and $2{\cdot}2\times10^8$ e.v. respectively, instead of at $3{\cdot}0\times10^8$ and $3{\cdot}7\times10^8$ e.v. for pseudoscalar mesons only. Now if the maxima occur at lower energies, which is the case when mesons of all polarization are produced, the absolute value of γ_1 at a mean energy 8×10^8 e.v. (which is the mean energy in Shutt's experiment) would be much smaller and the agreement would be apparently much better, as has been pointed out by Heitler and Peng.[23] But, in order to compare the theoretical values rigorously with experimental results, γ_1 and γ_2 should be integrated over the whole energy spectrum. If this is done the average value comes to 2·5 times the value of σ_1 and σ_2 obtained above for pseudoscalar mesons only. It is quite clear that if g^2 (= 1/9) is replaced by G^2 (= 1/3) in $\gamma_1/4\pi g^2$ and $\gamma_2/\frac{4}{3}g^2$, the values of γ_1 and γ_2 as obtained from the

curves in Fig. 2 would be trebled. But due to the shifting of the maxima to lower energies the final values of σ_1 and σ_2 are only increased by a factor 2·5. Thus both Shutt's experiment and the present experiment favour definitely the production of either pseudoscalar or transverse mesons rather than the production of mesons of all kinds of polarization.

Cross-section for pair production at low energies

We shall now make another estimate of the cross-section for the production of meson pairs from single mesons in the low energy region. We shall see that the experimental value at the low energy region shows a better agreement with theory.

In Table III in reference 9 the rates of two-meson multiples produced in 5 cm. and 10 cm. of lead are given as 4·63 per 100 hours and 2·04 per 100 hours respectively. From this we may say that at least (4·63−2·04), i.e. 2·59 meson pairs that are produced in 5 cm. of lead per 100 hours, are absorbed in the next 5 cm. The individual mesons of these multiples have a range greater than 2·2 cm., since they penetrate the lead plate inside the chamber. Expressed in percentage the above result is the following. At least 56% of the meson pairs that are produced in 5 cm. of lead have a range between 2·2 cm. and 7·2 mm. of lead. The energy corresponding to this range is $1{\cdot}6\times10^8\rightarrow2{\cdot}2\times10^8$ e.v. according to Rossi and Greisen.[24] Since the individual mesons of the meson pairs have an energy between 1·6 and $2{\cdot}2\times10^8$ e.v. the parent mesons responsible for these meson pairs would have an energy between 3·2 and $4{\cdot}4\times10^8$ e.v., assuming that conservation of energy holds good and the recoil nucleon does not take up any energy from the incident meson.

If we construct a graph from Table I, we find that the number of mesons lying in the energy interval $3{\cdot}2\rightarrow4{\cdot}4\times10^8$ e.v. is approximately 2·5% of the whole energy spectrum. Altogether seven meson pairs were produced in 5 cm. during a time of 151 hours. 56% of these, i.e. 3·92 meson pairs, on an average, have a range between 2·2 and 7·2 cm. The number of mesons of energy between 3·2 and $4{\cdot}4\times10^8$ e.v. which would excite our system in 151 hours is 2·5% of 3805, i.e. 95·1, for 3805 mesons of all energies get through 5 cm. of lead in 151 hours. Hence the fraction Δ of a meson that has converted into meson pairs in this energy region in 5 cm. of lead is

$$\Delta = \frac{3{\cdot}92}{95{\cdot}1}$$

Hence the cross-section for the production of meson pairs in the energy range $3{\cdot}2\rightarrow4{\cdot}4\times10^8$ e.v. is

$$\text{B.} \qquad \gamma_{2\,\text{exp}} = \frac{3{\cdot}92}{95{\cdot}1} \times \frac{1}{5\times6{\cdot}8\times10^{24}} = 1{\cdot}23\times10^{-27}\ \text{cm.}^2\text{/per nucleon} \pm 40\%.$$

The statistical error is high, for the number of meson pairs observed is not large. The theoretical value of γ_2 as obtained from Heitler and Peng's curve (Fig. 2) in this energy region is

$$\gamma_{2\,\text{theor}} = 1{\cdot}98\times10^{-27}\ \text{cm.}^2\text{/per nucleon,}$$

assuming that only pseudoscalar mesons are produced. If mesons of all polarization are produced, the mean theoretical value for the energy range quoted above will be $2{\cdot}2\times10^{-27}$ again pointing to a worsening of agreement. Here, however, the agreement between the theoretical and experimental values is much better than that for the whole energy spectrum. The theoretical value, though higher than the experimental value as before, is only 1·6 times the experimental value.

In making a comparison between theory and experiment here, the following point is to be noted. We have assumed that the difference between the rates of meson pair production in 5 cm. and 10 cm. lead gives the absorption of meson pairs in the region 5→10 cm. The percentage absorbed has been found to be 56. The amount of absorption is certainly greater than this, for some fresh meson pairs are also produced between 5 cm. and 10 cm., and these are included in the observed rate under 10 cm. of Pb. If these were excluded from the rate of meson pair production in 10 cm. this would give a larger value for the rate of meson pairs produced within 5 cm. and are absorbed between 5 cm. and 10 cm. of Pb.

Thus the value of γ_2 experimentally determined above represents the minimum value under the experimental conditions and the exact value would be nearer the theoretical value as given by Heitler and Peng.[11] It is, however, certain that the agreement is much better in the low energy region where the theoretical calculations are more precise.

The above conclusion is also supported by the results of another investigation carried out by the writer [25] under entirely different experimental conditions. In this investigation the scattering of mesons of energy lying between 1·5 and $2{\cdot}5\times10^8$ e.v. in two lead plates of thickness 2 and 4 cm. was studied. Following a method put forward by Shutt [21] the nuclear scattering cross-section of these mesons were found out to be

C. $$\gamma_1 = 1{\cdot}84\times10^{-26} \text{ cm.}^2\text{/per nucleon } \pm30\%.$$

The theoretical value of γ_1 corresponding to the mean energy 2×10^8 e.v. involved in this experiment, as given by Heitler and Peng's curves, is 2×10^{-26} cm.^{2}/per nucleon for pseudoscalar or transverse mesons. The agreement between the theoretical and experimental values in the low energy region is thus found to be quite good, though Shutt's [21] average value for the whole energy spectrum shows considerable discrepancy. We have found out above that the cross-section for the second order process of the splitting up of a meson into a pair shows the same features, namely, good agreement in the low energy region and considerable deviation when averaged over the whole energy spectrum.

Lastly we find from Table III of reference 9 that the average multiplicity or density of meson showers produced in 5 cm. is 2·4 and that in 15 cm. is 3·2. It is not possible to make a reliable quantitative comparison of the experimental data for higher multiplicities for the data are not sufficient for such a comparison. One point is, however, of significance. It will be seen from Heitler and Peng's [11] curves on the probabilities for higher multiplicities that the maxima for $n=2$ and $n=3$ occur at 4·2 and 5·8 μc^2 respectively. Thus the two maxima are separated by an energy interval of 1·6 μc^2 which corresponds to a range of about 10 cm. of lead. The two maxima experimentally obtained for meson showers of average multiplicity 2·4 and 3·2 are also separated by a lead thickness of 10 cm. This, we believe, is not merely a coincidence but points towards a rough agreement for higher multiplicities.

The author is much indebted to Prof. D. M. Bose for his kind interest and helpful suggestions.

Summary

In this paper the results of a previous experiment [9] have been utilized to find out the cross-section for the production of meson pairs by scattering of mesons in the nuclear field of protons and neutrons. An average value of the cross-section for whole energy spectrum of mesons as well as the value for a mean energy of about 4×10^8 e.v. have been determined. The theoretical value of the corresponding quantity for the whole energy spectrum has been obtained by numerically integrating Heitler and Peng's curve for the

cross-section, over the whole energy-spectrum of mesons as determined by Hughes. The agreement between theory and experiment is satisfactory in the low energy region whereas the theoretical value for the whole energy spectrum comes out to be four times the corresponding experimental value. The experimental value of the cross-section for the first order process of scattering into single mesons, as determined very recently by Shutt, shows almost exactly the same discrepancy with the theoretical value for the whole energy spectrum whereas the cross-section for single scattering, as determined by the author in a different experiment for low energy (2×10^8 e.v.) mesons, shows better agreement with the theoretical value. Thus it appears that the theoretical results are quite in accord with experiment in the low energy region while some modification in the theory is necessary to make the experimental results consistent with the theory for the whole energy spectrum. It is remarkable that any attempt in modifying the theoretical value of the first order process, so as to bring it nearer the experimental value as determined by Shutt, would automatically bring the theoretical value for the second order process nearer the experimental one as determined by the author. The whole result has been summarized in Table III.

TABLE III

Theoretical and experimental values of σ_1, σ_2, γ_1 and γ_2

Average for the whole energy spectrum		Author		For mean energy	Theoretical	Experimental	Author
Theoretical	Experimental						
$\sigma_2\times10^{28}$ cm.^{2} per nucleon.	49·5	6·55 (First value) ±10% 14·0 (Second value) ±10%	Shutt (*a*) and (*a'*) in text.	$\gamma_1\times10^{26}$ cm.^{2} per nucleon.	2×10^8 e.v.	2	1·84 ±30%	Sinha (C) in text.
$\sigma_2\times10^{29}$ cm.^{2} per nucleon.	19·4	5·4 ±25%	Sinha (A) in text.	$\gamma_2\times10^{27}$ cm.^{2} per nucleon.	$3{\cdot}8\times10^8$ e.v.	1·98	1·23 ±40%	Sinha (B) in text.

σ_1 and σ_2 are the values for scattering of single meson and for meson pair production by single meson, averaged over the whole primary meson spectrum.

γ_1 and γ_2 are the values of the same quantities for mean primary meson energy 2×10^8 e.v. and $3{\cdot}8\times10^8$ e.v. respectively.

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XII. STUDIES ON THE MECHANICAL PULSATION AND RESPIRATION IN THE MOTILE LEAFLETS OF *DESMODIUM GYRANS*

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Introduction

§1. *Desmodium gyrans* is a papilionaceous plant with trifoliate leaves, of which the terminal one is large and the two lateral ones are small. The lateral leaflets have small pulvinules; they are seen to execute pulsatory movements of periods varying from one to four minutes depending upon the season and the age of the leaflet. The gyratory motion of a leaflet can be recorded by attaching its tip to two oscillating recorders, arranged to record movements at right angles to each other and to the length of the leaflet.[1] This activity can also be observed when the petiole is detached from the living plant and its cut-end inserted through a cork to a small glass U-tube filled with tap water (filtered river water).

The pulsatory activity of such motile leaflets were the subject of many investigations by Sir J. C. Bose, and the results obtained are recorded in detail in his three earlier monographs *Plant Response* (1906), *Comparative Electrophysiology of Plants* (1907) and *Researches on Irritability of Plants* (1913). Previous to Bose, Darwin,[2] Jost [3] and Pfeffer [4] had made a few observations on the nature of the pulsatory movement and on the influence of a few environmental agencies on it. The effects of temperature in altering the amplitude and period of oscillation, the effect of CO_2, alcohol, ether, chloroform and other chemicals on the pulsatory activity were investigated by Bose. He also found that electric pulsations of double the frequency accompany the mechanical one. In fact a great deal of similarity was found between the behaviour of such leaflets and that of animal hearts. It was also noticed that when the pulsatory activity stops, it can be temporarily revived by the application of electric stimulus, of light, and of sugar solution.

According to Bose, the energy which expresses itself in pulsating activity is derived by the plant either directly from immediate external sources or from the excess of such energy already accumulated or held latent in the tissue. He showed that when this storage is exhausted, the so-called spontaneous movement ceases and in that condition of standstill the rhythmicity of the leaflet can be revived by an application of fresh stimulus. In this condition of run down of energy the leaflet behaves quite like the leaves of other sensitive plants, such as *Mimosa* or *Biophytum* which also give single or multiple responses under stimulus.

Bose also studied the depletion of energy of the detached leaflets under isolation from all accession of energy from outside. He found that the persistence of the rhythmic activity under the condition is dependent on the vigour of the specimen, that is to say on the storage of energy in the tissue. He found that a vigorous specimen exhibited activity for more than twenty hours, while in a less vigorous specimen this activity came to stop at the ninth hour. From all these experiments he has drawn the conclusion that the movement of *Desmodium* is not absolutely spontaneous but is dependent on stored up energy. But he has not stated the actual form of energy required in the pulsating activity of *Desmodium*. It may be reasonably conceived that the energy required in the pulsating activity is not fundamentally different from the energies required for other physiological

functions of the plant. But though the energy source may be the same in all functional activities of the plant, it may undergo different transformations to be utilized in different activities. Since 1939, we have taken up the investigation of the source of energy of the pulsatory activity.

This account of the investigations has been divided in two parts. In the first part an account is given of how 24-hour photographic records of the pulsations of detached leaflets of *Desmodium*, with cut-ends dipped in tap water, have been obtained. At the same time automatic records were also kept of the temperature and light intensity variations to which the leaflets were subject. During the course of the investigation it transpired that the energy of the mechanical pulsations was derived from the breakdown of carbohydrates formed in the leaflet by photosynthesis during daylight. The validity of this inference was tested by making the leaflet imbibe glucose by dipping its cut-end in one per cent glucose solution, and observing how the resulting storage of carbohydrate in the leaflet affected its pulsation.

Since there is an intimate relation between carbohydrate breakdown in plants and respiration, in the second part an account is given of an apparatus, which was devised to record simultaneously on the same smoked glass plate, the pulsatory and respiratory response of a *Desmodium* leaflet, to find out how they were both influenced by different environmental conditions, which include the effect of light and darkness and of various nutrients. The results obtained have been discussed in light of recent theories of carbohydrate breakdown mechanism in plants.

PART I

Mechanical Pulsations in Desmodium leaflets : Source of Energy

§2. For the purpose of our investigation it was considered necessary first to obtain the diurnal records of mechanical pulsations of the leaflets under different age conditions. It has been previously mentioned that the continuance of the pulsatory activity under condition of isolation depends on the vigour of the specimen; in a vigorous specimen it may continue for more than twenty hours, while in a less vigorous one it may stop within nine hours. Greater vigour no doubt indicates larger storage of energy, but the condition of the leaflet on which this storage is dependent has not been investigated. For taking the diurnal record of the pulsatory activity of the leaf an automatic photographic device has been employed, whose description is given below. For observation of the influence of light and temperature automatic recording arrangements for recording both of these important environmental factors were also made.

APPARATUS

§3. *Plant Recorder.*—The smoked-plate recorder described by Sir J. C. Bose for recording *Desmodium* pulsations was not found to be suitable for continuous diurnal recording. Therefore a specially devised recorder was constructed for recording photographically the pulsations of *Desmodium*. A small light mirror is attached to the horizontal axis of a well-balanced delicate lever, so that the mirror undergoes rotation, due to up and down movements of the lever induced by the attached leaflet. The mirror and the lever are so adjusted that when the lever is in the horizontal position, the mirror faces downward. A beam of light from a small incandescent electric lamp is directed toward the mirror at a suitable angle and the reflected spot of light, which moves in a vertical plane, is converted into a horizontal movement by means of a totally reflecting glass

prism, suitably placed below the mirror. The whole arrangement is enclosed in a blackened metal box having a fine slit through which the lever projects out, and the channel through which the reflected light is transmitted to the photographic recorder. The photographic recorder which is also enclosed in a box consists of a horizontal drum on which the photographic paper is attached, and a clockwork arrangement which rotates the drum uniformly once in twenty-four hours. A fine slit with a cylindrical lens is placed in front of the drum for producing a sharp image of the spot of light. The above-mentioned channel of the metal box is coupled with the slit of the photographic recorder so that the whole arrangement becomes light proof. The complete apparatus is diagrammatically represented in Fig. 1.

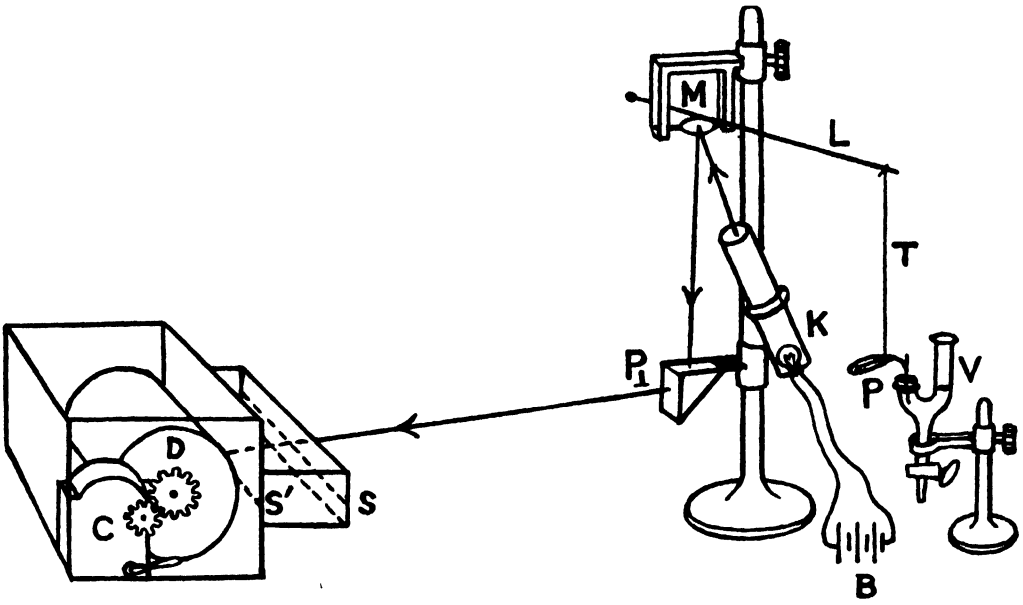


FIG. 1. Diagrammatic representation of 24 hours' recorder for recording the pulsation of *Desmodium* leaflet.

P, *Desmodium* leaflet mounted in water in U-tube V; T, fine silk thread attaching the leaf to the lever L, by the up and down movement of which a light mirror M, fixed on its axis, undergoes rotation in a vertical plain; B, electric battery for lighting the incandescent lamp K, the rays from which after being reflected from the mirror is again reflected through the prism P_1, and thus converts the vertical movement of the spot of light to a horizontal one; the ray of light then passes through the slits S and S', and falls on the photographic paper wrapped round the drum D; C, clockwork arrangement for rotating the drum once in 24 hours.

§4. *Photo- and Thermographs.*—For recording diurnal variations of light and temperature, an oscillating-plate recorder carrying two separate levers was used. The variation of temperature induces a curvature of a bi-metallic strip made of brass and iron; this curvature is recorded by the upper lever of the recorder attached to the strip. For recording light variations, a Weston photo-cell was used. The terminals of the photo-cell are attached to a vertically placed micro-ammeter; the pointer of the ammeter is attached with a fine silk thread to the lower lever of the recorder, which records the current changes of the micro-ammeter and consequently the light variation. The amount of light was measured by means of a barrier type photo-electric cell coupled with a direct

current vacuum tube amplifier and a milli-ammeter, the latter being calibrated in terms of foot-candle. The smoked plate oscillates once in 15 minutes; thus the interval between two dots is 15 minutes. The photo-cell and the micro-ammeter with the recording device are diagrammatically represented in Fig. 2. A specimen record of the diurnal changes of

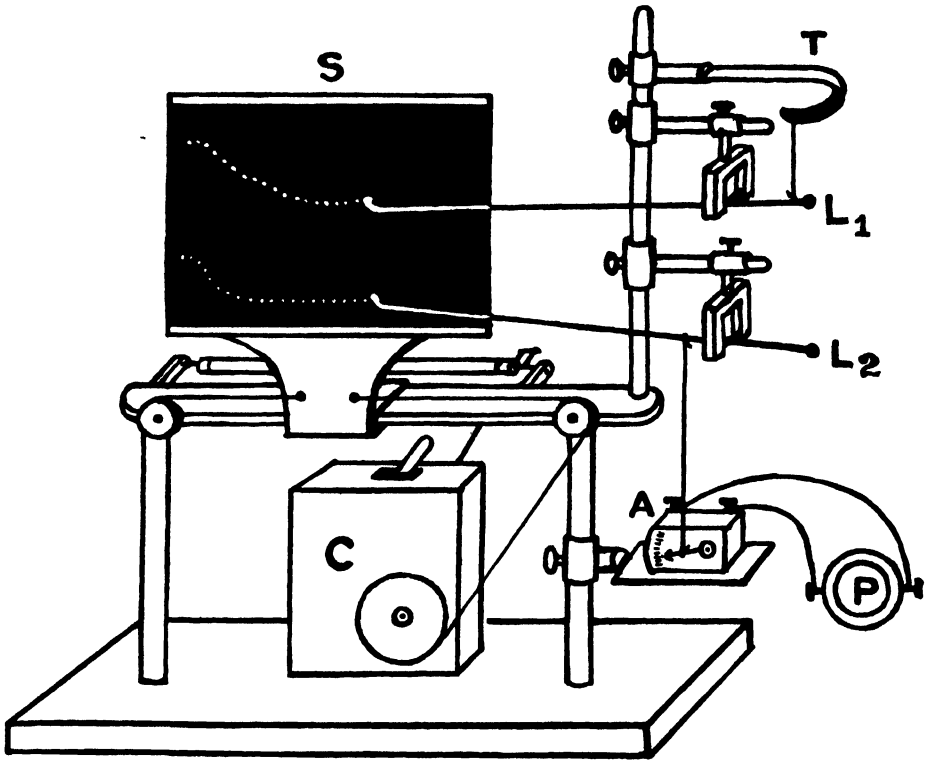


FIG. 2. Photo- and Thermograph for recording diurnal variations of light and temperature. L_1, lever attached with the bi-metallic strip T, for recording temperature variations; L_2, lever attached with the pointer of the milli-ammeter A, for recording the light variations indicated by the photo-cell P; S, smoked glass plate; C, clockwork arrangement for moving the smoked glass plate from right to left.

light and temperature obtained by the above-mentioned device is given in Fig. 3.

EXPERIMENTAL PROCEDURE

§5. A petiole of *Desmodium gyrans*, with the terminal leaflet and two lateral leaflets, is detached from the plant growing in the Institute garden, and the cut-end is fixed in a glass U-tube containing tap water with a piece of split cork, care being taken not to apply excessive pressure on the petiole. The glass U-tube is fitted with a stop cock at the lower side, so that the solution contained in it can be changed when required. The terminal leaflet and one of the side leaflets are discarded with a sharp knife and there remains only one side leaflet of which the record is to be taken. The U-tube with the plant is fixed on an adjustable stand and placed below the projecting lever of the plant recorder described above. One end of a fine silk thread is attached to the leaflet with a minute quantity of shellac varnish, the point of attachment being approximately one-fourth the length of the leaflet from the pulvinule. The other end of the thread is attached to the

lever end above the leaflet. After a period of one hour's rest the leaflet is allowed to record its pulsations for twenty-four hours. At the end of twenty-four hours the tap water in

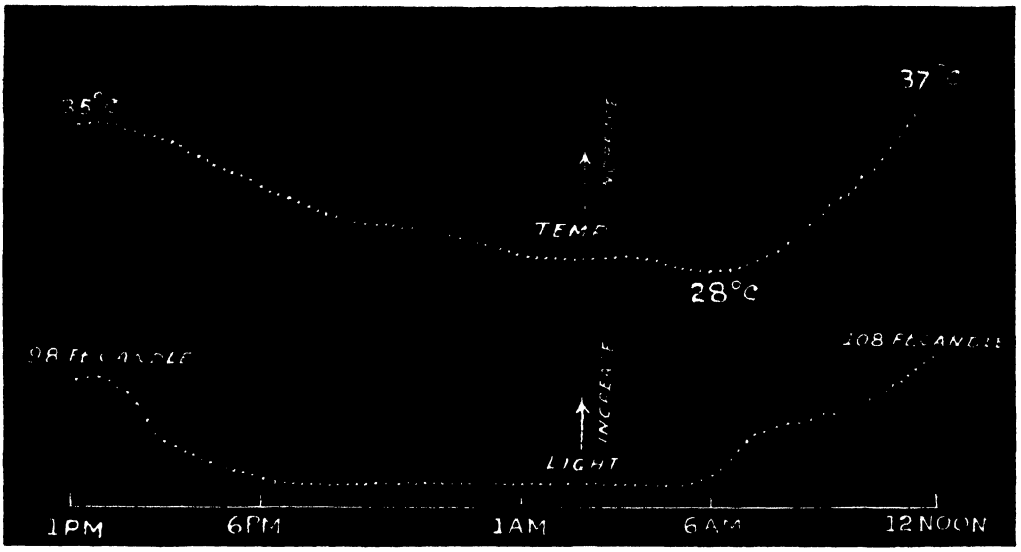


FIG. 3. A specimen record of the diurnal variations of light and temperature. Interval between two dots is fifteen minutes.

the U-tube is replaced by fresh water or it is replaced by any other solution as the case may be. And the recording is continued again for another twenty-four hours.

EXPERIMENT 1

§6. *Pulsatory activity of young, medium and old leaflets.*—The leaf at the top of the branches, which were fully open but yet very tender and light coloured, were considered as young leaf. Mature green leaves usually beyond fourth or fifth from the top were taken to be medium leaves. Leaves in the lower regions of the branches which were deep coloured and sluggish in movement were considered as old leaves. Typical diurnal records of the pulsatory activity of the leaves in the three stages mentioned above, are given in Fig. 4.

In the record A (Fig. 4), which represents the pulsation of a young leaf, the pulsation is seen to stop at an early hour after nightfall and to revive in the morning. The pulsation of a medium leaf represented in the record B, is found to continue through day and night. At night, of course, the frequency of pulsation diminishes comparatively as indicated by the widening of the individual pulses. The pulsations of an old leaf, in the record C, seem to be sluggish and irregular though it continues throughout day and night.

It has been found from a large number of experiments that a detached young leaf from a vigorously growing plant, when placed with the cut-end of the stem in ordinary tap water, lives from fifteen to twenty days with its normal pulsatory activity in no way impaired. The successive diurnal records of such leaves were identical to the one given in Fig. 4A, with similar nocturnal stoppages. In a medium leaf under similar conditions the pulsation continues for one or two days without any break and afterwards it stops for good. After the stoppage of pulsation, the leaf, however, lives for a few days more.

In the old leaf under detached condition the pulsation seldom continues beyond twenty-four hours.

The nocturnal stoppage in the young leaf indicates depletion of energy accumulated during the day by photosynthesis. The absence of nocturnal stoppage in the medium or old leaf can be accounted for by its capacity for greater storage of energy during daytime, due probably to the abundance of its chlorophyll content. The widening of pulsation, i.e. decrease in frequency at night may be attributed to the effect of lower temperature at night (Bose). The comparatively short life of the medium leaf under detached condition remains yet to be explained.

§7. *Glucose as a substitute for photosynthetically stored energy.*—The result of Experiment 1, points to the conclusion that the nocturnal stoppage of pulsation in young leaf is due to the depletion of energy accumulated in the daytime. But the accumulation and depletion of energy requires further elucidation for experimentally establishing the hypothetical conclusion. A preliminary discussion of the results found by different workers on the metabolism of leaves in relation to light may be helpful at this stage. It is known that plant synthesizes carbohydrates, nitrogen compounds such as proteins, amides and amino acids, other vegetable acids like lactic, fumaric, succinic, malic, tartaric and citric, aromatic compounds like tanins, anthocyanins and glucosides. Although it has not been possible yet to make positive assertions about the stages in the synthesis of different substances, different workers agree in the view that of these metabolites only carbohydrates are produced during photosynthesis, and most of the other substances result from the metabolism of carbohydrates and are not themselves photo-biochemical products. Of the carbohydrates again, only the hexose or the reducing sugar requires light energy for its formation and this primary metabolite undergoes condensation to yield higher carbohydrates without further intervention of light energy. The earlier convention of regarding the primary metabolite was, however, quite different. Experimental results on the diurnal changes in the concentration of different carbohydrates obtained by Parkin,[5] Gast,[6] Miller,[7] etc., in different leaves indicate that cane sugar considerably increases in light and decreases at night, while the concentration of the reducing sugar does not fluctuate in a manner that can be significantly correlated with light intensity. The earlier workers interpreted these results as showing that cane sugar is the first carbohydrate to be formed in photosynthesis, and hexoses are derived from sucrose by hydrolysis. Later Weevers [8] investigated the problem of the first sugar formation in photosynthesis, by determining the production sequence of carbohydrates during the process. He experimentally showed that carbohydrate appears in the following order: Hexose, cane sugar, starch, and concluded that hexoses are the precursors of cane sugar. At present this view is widely held. It is now supposed that cane sugar or starch cannot be synthesized until, as a result of photosynthesis, a certain minimal critical concentration of hexoses has been attained. The further production of hexoses above this critical amount then results in the synthesis of cane sugar and starch. Consequently the concentration of hexose remains approximately constant, while the concentrations of cane sugar and starch vary directly with the rate of photosynthesis. Thus in the accumulation of energy rich substances hexose plays an essential part; a portion of hexose formed may accumulate as such, and the excess amount undergoes condensation to yield higher carbohydrates or to produce other complex compounds which act as source of potential energy to the plant.

In the liberation of energy by respiratory oxidation, hexose is finally broken down into CO_2 and water. When the respiratory substance is a higher carbohydrate it has to be hydrolyzed before being finally utilized in respiration.

Further, in the normal life of green plants carbohydrates are first tapped as respiratory substrate. Blackman [9] has termed this carbohydrate respiration as floating respiration. His experiments gave the first clue that when the supply of these diminished, the floating respiration gradually declines. After that the residual respiration or protoplasmic respiration as Blackman termed it, remains more or less constant for a period, indicating that other substances in the protoplasm may be oxidized when shortage of carbohydrates become acute. This, however, does not exclude the possibility that protoplasmic respiration proceeds in addition to floating respiration under normal condition even when the cells are provided with an ample supply of carbohydrates. Deleano [10] showed by measuring the different respiratory substrates during respiration under starved condition in the detached leaves of *Vitis vinifera*, that during a period of one hundred hours, all the CO_2 produced came from carbohydrates. The proteins gave rise to soluble organic products and ammonium salts only when the leaves were starved in darkness for over four days. Deleano inferred that although the leaves possess the oxidizable proteins and other nitrogenous substances they are consumed only under conditions of extreme want; he maintained that under natural condition only carbohydrates are consumed. Yemm [11] by measuring the different carbohydrate fractions during the process of respiration in barley leaves placed in the dark, showed that glucose is an oxidizable carbohydrate and so also is fructose, which is still more readily oxidized.

Thus in all biochemical processes accompanying energy metabolism in plants hexose or the reducing sugar occupies a key position. It can, therefore, be most reasonably presumed that if a plant is continually supplied with a reducing sugar it can derive all its energy requirements from it, even in a condition when its synthesis is stopped in the plant due to the absence of light energy. Therefore in our present investigation, if the nocturnal stoppage of pulsation be due to the depletion of carbohydrate reserve in the leaflet it can naturally be expected to revive it by the supply of reducing sugar. Taking this view into consideration an attempt was made to find out if the nocturnal depletion of energy can be replaced by supplying the leaf with glucose.

Experiment 2

§8. *Effect of glucose on the pulsatory activity of Desmodium gyrans.*—Normal records of pulsations of *young* detached leaflet with the cut-end of the stem dipped in tap water, were taken for two to three days, after which the water was replaced by a 1% solution of glucose. Fortunately the experiment yielded some very convincing results. The nocturnal stoppage in the diurnal records of pulsation was found to disappear either on the first day of application or on the second day; the pulsation became continuous throughout day and night. Freshly prepared solutions were applied everyday to avoid the effect of alcoholic fermentation. When the glucose solution was withdrawn and substituted by tap water again the nocturnal stoppage was found to reappear. In some cases, the after-effect of glucose was found to persist for a few days even after the stem was withdrawn from the glucose solution and the normal nocturnal stoppage did not reappear until the specimen had been kept in tap water for a few days. This occurred specially in cases where the application of glucose was prolonged. The effect of the application and withdrawal of glucose solution has been repeatedly tried on several specimens, in every case with identical results. A typical record is given in Fig. 5, which shows the effect of glucose on the pulsation under diurnal variation of light.

In Fig. 5, the first two days' records A and B, represent the normal behaviour of the detached pulsatory leaflet with the cut-end dipped in tap water. The nocturnal

stoppage is well marked in both of them. The records C and D were obtained when the cut-end of the stem is dipped in 1% glucose solution. In the record C, i.e. on the first day of glucose administration, the duration of nocturnal stoppage is seen to be reduced to a very short period, while in D, i.e. on the second day of glucose application the nocturnal stoppage has disappeared; the pulsation is continuous throughout day and night. The record E is taken after the withdrawal of glucose. This, however, is not the immediate record of withdrawal. The effect of the application of glucose persisted for four days after its withdrawal and E, is the record on the fifth day after the withdrawal. Glucose was again applied to the same specimen on the fourteenth day of experiment, and the nocturnal stoppage completely disappeared on the second day of application, as is seen in the record G. The glucose solution was again withdrawn and in the next day's record H, the effect of glucose is seen to be partially continued. But the normal nocturnal stoppage appeared this time, on the second day after the withdrawal of glucose as shown in the record I. The detached leaflet under investigation continued to live, under experimental conditions, for nineteen days.

The results of the above experiment leave no doubt that the nocturnal stoppage is due to the run down of energy photosynthetically stored in the leaf during the daytime and that the depleted energy can be resupplied to the leaf by absorbing glucose. That glucose can be retained in excess of the immediate requirement in the tissue of the leaf either as it is, or in a transformed condition, is evident from the continuance of the pulsation at night even after the withdrawal of glucose.

EXPERIMENT 3

§9. *Effect of glucose on leaf kept in complete darkness.*—In the previous experiment it has been shown that under normal diurnal variation of light and temperature the nocturnal stoppage of pulsation is annulled by the application of glucose. Consequently glucose can be regarded as a substitute for the photosynthetically stored energy required for pulsation, the depletion of which causes the stoppage at night. But it cannot be called a complete substitute for the energy of pulsation derived from light, unless it can supply the same continually in total absence of light. The effect of glucose was, therefore, recorded by keeping a *young* leaf, in which the pulsation ceases at night, in complete darkness for several days. After taking the normal record of pulsation for two days the chamber was completely covered with a light-proof box with adequate ventilating arrangement, and glucose was administered. The box was fitted with two tubes, one at the lower and the other at the upper side, the latter being connected with an exhaust pump worked by running tap water, for continuous circulation of air inside the box. It has been found that under complete darkness the pulsatory activity is continued for a day or two by the administration of glucose after which it stops for good. The pulsations in darkness are considerably decreased in amplitude as well as in frequency in comparison to normal ones. It has been found that when the darkness is continued for two to three days, the leaf does not revive when brought back to normal light. A typical record is given in Fig. 6. The first day's record represents the normal diurnal record of pulsation. The second and third days' records were taken in complete darkness under the application of glucose. The continuance of the pulsatory activity throughout day and night in darkness is noticeable. On the second day in darkness the pulsation stopped after 18 hours, after which the leaf did not recover even when brought back to light.

From the above experiment it is evident that glucose can act as a substitute for the photosynthetically stored energy utilized in the pulsatory activity of the leaf. But under

continuous darkness, the leaf does not survive over 48 hours, which shows that sunlight is necessary to the plant not only for photosynthesis but for other physiological processes.*

Discussion

§10. Up till now, it is not known why the lateral leaflet of *Desmodium gyrans* execute autonomous pulsatory movement and whether this movement is necessary at all for the economy of the plant. The only suggestion given by Bose is that the so-called spontaneous pulsation is not self-originated, but is due to an antecedent external stimulus. The persistence of autonomous activity is thus dependent on the amount of stimulation to which the plant had been previously subjected; the energy supplied by the environment becomes as it were, latent in the plant, increasing its power of work. The results of the experiments reported in the present paper, show that the external stimulus postulated by Bose is the light incident on the leaflet and the energy of stimulation is stored up as carbohydrate synthesized in the leaf under light absorption. The difference in behaviour of young and older leaflets regarding their pulsatory activity, is also interesting. The young leaf stops pulsating at night and resumes it again at daybreak, whereas, the older leaf goes on pulsating throughout twenty-four hours without any cessation at night. The leaf observed by Bose, which could pulsate for more than twenty hours when kept in darkness, was probably a mature leaf. The older leaf, which has a deeper green colour than the young one, due to a larger chlorophyll content, can presumably manufacture more carbohydrates by photosynthesis during the day than a young life. This carbohydrate being more than sufficient for its energy requirement during daytime, the excess is utilized at night; hence the ceaseless pulsation throughout day and night. On the other hand, the young leaf, due to its smaller chlorophyll content forms lesser quantity of carbohydrate which only suffices for its energy requirement during daytime, and therefore, the pulsation stops at night. Now the question arises as to why the older leaf with greater storage capacity of carbohydrate stops pulsating within two to three days, while the young leaf with a lesser capacity can pulsate for as long as twenty days. According to Bose, the greater the subtonic condition of the specimen, the stronger is the stimulation required to induce a response in it. Hence the older leaf, due to advance in age, is evidently in a more subtonic condition than the younger one, therefore, absorption of more light energy is required to induce pulsating response in it than that required by the younger one. The specimens used for experiments were collected at noon from the open garden of the Institute, i.e. after the leaves had full exposure to direct sunlight, and the experiments were conducted in a corridor with diffused sunlight; consequently the experimental leaf received more sunlight on the first day of the experiment than on subsequent days. Hence the leaves had more carbohydrate reserve on the first day than on the subsequent days. That the above assumption is plausible is evident from the first two days' normal record of pulsation of the young leaf; it will be noticed that its pulsation on the first day persisted till eleven o'clock at night whereas, on the second day it persisted only up to half past nine. That the light intensity greatly affects the pulsatory activity of the leaf

* It appears that detached belladona leaves fed with ammonium sulphate plus sucrose in the dark increase their amount of alkaloid per leaf more vigorously than leaves kept on water or sucrose solution only. Similar leaves showed no protein accumulation during such period (W. O. James, Biosynthesis of the Belladona Alkaloids, *Nature*, **158**, 654, 1946).

Apparently protein formation in leaves requires the absorption of light; this may have some bearing on the above reported failure of young *Desmodium* leaflets to revive its pulsatory movements when brought to light after prolonged stay in dark.

was noticed in some of the records of the young leaf—not mentioned in the body of the paper. In one such experiment a young leaf ceased to pulsate continuously for three days, due to persistent cloudy state of the sky. When the sky cleared up the leaf began to pulsate again as before. Therefore, the failure of the older leaf to continue pulsation for longer periods, may be attributed to the lesser light intensity available under the experimental condition. Had the experiment been performed in the open space of the garden with full exposure to direct sunlight on the older leaf, we could expect continuance of its pulsation for a longer period.

That the greater chlorophyll content of the older leaf is responsible for the absorption of a larger amount of energy and thereby for maintaining the continuity of pulsation at night, is convincingly proved from an analysis of the continuous normal records of different young leaves. The young leaf which is light green at the start gradually begins to take deeper colour as the days pass, and the stoppage of pulsation after nightfall is gradually delayed after a few days.

The diurnal variation of light and darkness has been found to influence the pulsation of *Desmodium* more profoundly than that of the temperature. The effect of variation of temperature has been previously studied by Darwin,[2] Bose and others. Darwin found that at 52°F. the leaf totally ceased to move while at 100°F. the movement was rapid and still more rapid at 105°F. Bose showed from a number of experiments that the pulsation of *Desmodium* slows down and finally stops at a thermometric minimum which was about 63°F.; the rise of temperature on the other hand increase the frequency of pulsation up to 107°F., but the amplitude becomes so small that the pulsation appeared to have come to a stop. In our present experiment the general diurnal variation of temperature was found to be between 82°F. and 92°F., which according to both Bose and Darwin, is favourable for pulsation. Moreover, had the stoppage been due to the lower temperature at night it could not be checked by the application of glucose only. Slight decrease of frequency of pulsation at night can, however, be correlated with the lower temperature at night.

The experiments just mentioned were conducted under normal variation of light and darkness. Now the question may arise, whether glucose can provide all the energy requirements if the plant is kept in darkness even in daytime. Experiment 3, shows that by the application of glucose in complete darkness the pulsatory activity is continued for about two days though in a feebler condition. But the leaf stops pulsation and dies even when it is brought to normal condition again. This shows that though glucose may supply the energy requirements of the plant, light is also absolutely necessary for the vital activity of the plant. In this connection the work of Robbins [31] may be mentioned. He found that the moss plant can absorb and utilize different sugars in dark but light is necessary for continued growth of the plant.

§11. From Bose's own investigations evidence can be obtained which shows that the pulsations of *Desmodium* leaflet is based upon a temperature dependent chemical reaction. H. Hoagland [12] cites a large number of such temperature dependent spontaneous rhythmic activities in living organisms, e.g. frequency of chirping of crickets, flashing of fireflies, heart beat of Limulus, velocity of progression of ants, etc. This rate of variation with temperature can be represented by Arhennius's equation $V = Ze^{-\frac{\mu}{RT}}$; where V is velocity of the underlying chemical reaction, which controls the rhythmic process. A rise of temperature of 10° more than doubles the rate of the chemical process, which is represented as ($Q_{10^\circ} = 2-3$). The following data are taken from Bose's *Motor Mechanism*

in Plants (p. 263) on the variation in the rate of pulsation of *Desmodium* leaflet with temperature :—

Temp. °C.	23°	28°	33°	38°
Frequency per hour ..	12·7	25	33	48

We find Q (38°−28°) = 1·9 and Q (33°−25°) = 2·5; which are of the expected order of magnitude, when the pulsation is due to a temperature dependent chemical reaction. No satisfactory answer can at present be given, as to how a continuous chemical reaction can give rise to pulsations. Rashevsky [18] has illustrated the possibility of coupled chemical reactions in living cells which can under certain conditions have a periodic character. He considers the example of the breakdown of glucose in cells to hexose diphosphate, when in contact with adenyl phosphate. It is well known that the energy of contraction of myosin muscle fibrils is supplied by such a phosphorylation reaction. Due to different rates of production and breakdown of these two substances, there will be under certain conditions a periodic variation in their concentrations even when the supply of glucose is so large that there is no appreciable change in its concentration within the cell. Such a reaction may be taken as model of the periodicity in chemical change underlying the mechanical pulsation of *Desmodium* leaflet. Another view is to consider such pulsations to be of the nature of relaxation oscillations, in which the supply of chemical energy is continuous, but the pulsations are due to some blocking effect induced in the tissue after the passage of an excitation through it.

PART II

RESPIRATION OF THE PULSATING LEAFLET OF DESMODIUM

§12. *Introduction.*—We have shown in the previous part that hexose, either manufactured in a motile leaflet of *Desmodium gyrans* by photosynthesis, or absorbed through its cut stem from a dilute glucose solution is utilized in the maintenance of its mechanical pulsation. We know that a breakdown of hexose in plant tissue, will supply energy for the synthesis of other plant products, etc. Measurement of the respiratory quotient gives some insight into the nature of the end products, e.g. if the ratio is unity, then the hexose is completely converted into CO_2 and H_2O.

We give in the next paragraph a short account of our present knowledge of the processes involved in the synthesis and breakdown of hexose in plant organisms, and then indicate how these results have been used to formulate our scheme of investigation on the relation between pulsation and respiration in a single motile leaflet of *Desmodium gyrans* under different experimental conditions. In a subsequent para we describe the apparatus used by us for the simultaneous recording of mechanical pulsation and respiration of a single leaflet, under different experimental conditions. The results of these experiments are discussed in the last paragraph.

§13. *Mechanism of hexose synthesis and breakdown in green plants.*—There is a large volume of evidence which shows that in animal organisms the energy required for muscular or other mechanical activities, the maintenance of temperature, and the synthesis of fats, proteins, etc., is supplied by either a total or partial dissimilation of glycogen stored up in the tissues. This glycogen represents the polymerization of glucose by the organism from absorbed food materials. In chlorophyll containing plants, as a result of a partly photochemical process in which through the intervention of chlorophyll, in a manner

not clearly understood at present, hexose is formed according to the following schematic reaction.[14]

$$CO_2+2H_2O \xrightarrow[\text{chlorophyll}]{\text{light}} (CH_2O)+H_2O+O_2;$$

where (CH_2O) is the resulting organic compound synthesized. Investigations described below show that photosynthesis is a partly photochemical process in which, with the assistance of specific hydrogen donors, which in the present case is H_2O, CO_2 is reduced to organic matter. In certain kinds of bacteria photosynthesis takes place without the evolution of oxygen, the specific hydrogen donor in some cases being SH_2, and the corresponding reaction is $CO_2+2H_2S \longrightarrow (COH_2)+H_2O+2S$.

According to this concept photosynthesis is an oxidation reduction process, induced by light and with the participation of special pigments, in which CO_2 is the final acceptor, while a variety of substances can serve as hydrogen donors. In green plants the evolution of O_2 during photosynthesis, results from the exclusive use of H_2O as donor. This view is supported by investigations of Rubens and co-worker, Winogradov and Teis,[14] using as trace element isotopes of O^{16}. They observed the following reactions :—

$$CO_2{}^{16}+2H_2O^{18} \longrightarrow (CH_2O^{16})+H_2O^{16}+O_2{}^{18}$$
$$CO_2{}^{18}+2H_2O^{16} \longrightarrow (CH_2O^{18})+H_2O^{18}+O_2{}^{16}$$

At present several instances are known in which the conversion of CO_2 into organic substances is accomplished by different organisms without the interference of light, and one of the most exciting discoveries of recent years has been the demonstration that in certain nonphotosynthetic organisms, the synthesis of organic substances from CO_2 is achieved through the mediation of phosphorous compounds which are characterized by a high degree of instability. It will be shown later that energy rich unstable phosphorous compounds like adenosine triphosphate (ATP) play an important rôle in the successive enzymatic breakdown (or synthesis) of glucose (from its lower intermediates) both in animal and plant organisms. Hence there is more than a remote possibility that these phosphorous compounds play an essential rôle in photosynthesis.

§14. Just as the formation of hexose by photosynthesis may be regarded as due to the successive reduction of CO_2 molecules by transference of hydrogen from donors, so breakdown of glucose may be regarded as an oxidation of the molecule, which acts as the hydrogen donor; the ultimate end product may be represented as follows:—

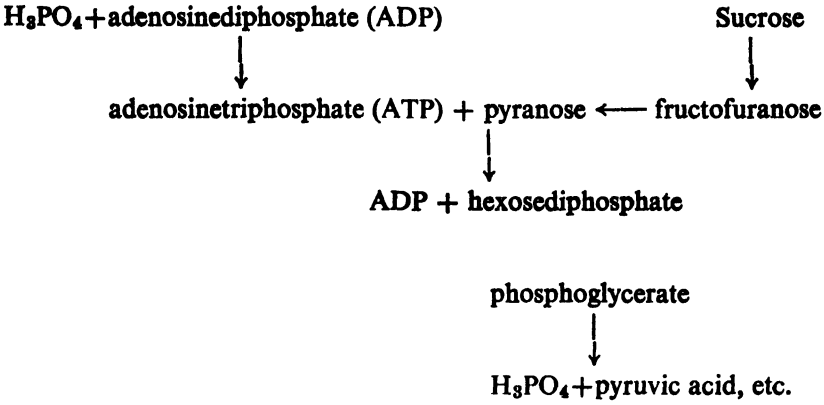
$$C_6H_{12}O_6+6O_2 \longrightarrow 6CO_2+6H_2O$$

We have a more detailed information at least of a part of the successive steps by means of which a transference of hydrogen from the glucose substrate takes place. In animal tissues the first part of the dissimilation of glycogen takes place through a chain of anaerobic reactions during which hexose is converted to triose leading to the formation of pyruvates; intermediate energy rich phosphorylated compounds like glucose-1-phosphate, glucose-6-phosphate, hexose diphosphate, phosphoglycerates, phosphopyruvates are formed. All these phosphorylation reactions, except that of glucose-1-phosphate, which requires the help of phosphorylase, are brought about by the successive breakdown and reformation of Adenosinetriphosphate (ATP)→Adenosinediphosphate (ADP)$+H_3PO_4$→Adenylic acid $+2H_3PO_4$. There is a large amount of evidence to show that either myosin (which forms the contractile element in skeletal muscles) or a substance strongly adsorbed in it, acts as the enzyme responsible for the breakdown of ATP→ADP. Investigations by Engelhardt [27] *et al.*, Needham [28] *et al.*, make it very probable that the reaction which is capable of

supplying free energy for the contraction of myosin fibrils in muscles, is closely associated with the breakdown by AT phosphotase of ATP to ADP and phosphate.

Investigations on the mechanism of hexose breakdown in plant tissues has not been so extensive or detailed. It is generally accepted that there is an intimate connection between the aerobic and anaerobic respiration; the first stages in the two processes being the same and consists in the breaking down of intermediate products, which in the presence of oxygen are oxidized to CO_2 and H_2O, and in its absence to CO_2 and ethyl alcohol.

The mechanism of glycolysis, the name given to the first common stage, chiefly in shoots of barley has been made the subject of special studies by James [15] and a number of collaborators. The successive steps in glycolysis has been represented by them by the following diagram



It will be noticed that up to the production of pyruvates, the proposed mechanism of glycolysis appears to be very similar to that in animal tissues. This is an illustration of the fundamental unity in the chemical behaviour of living organisms, that the formation of a certain product, by no matter what organism, involves very similar if not identical step reactions, from which it is assumed that the steps involved in the synthesis and breakdown of hexose in *Desmodium* should be similar to that found in barley shoots.

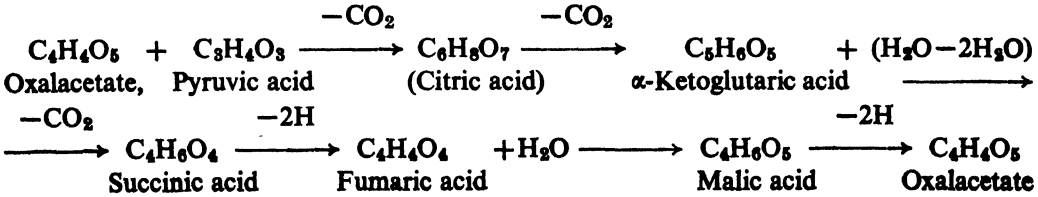
We find that the aerobic breakdown of hexose can be represented as taking place in two stages, in the first of which it is converted to pyruvic acid

$$C_6O_6H_{12} \longrightarrow 2C_3H_4O_3+4H$$

representing the transfer of 4H atoms from the substrate glucose to hydrogen acceptors. The next step represents the decarboxylation of pyruvic acid, according to the schematic representation,

$$2C_3H_4O_3+4H \xrightarrow{+6O_2} 6CO_2+6H_2O.$$

The successive steps by which this reaction takes place has not been well established. One scheme which has been proposed by Krebs is given below.[16] It starts with a combination of oxalacetic acid with pyruvic acid, to form citric acid and leads through a chain of cyclic reactions again to oxalacetate



The first part of the cycle is associated with the decarboxylation of the substrate, while the second part coincides with what is known as the Szent Gyorgi cycle. The functions of succinic, fumaric, malic and oxalacetic acids are quite different in the two cycles. In the Krebs cycle these acids are intermediates in the oxidation, whereas according to the Szent Gyorgyi theory they merely function as hydrogen carriers.

As reviewer has remarked 'Krebs offers a logical and picturesque path way for the complete oxidation of carbohydrate'. The citric acid cycle is not the only cyclic oxidation mechanism proposed to explain the oxidation of pyruvic acid, and other schemes have also been suggested.

We are now in a position to state the theoretical considerations underlying the investigations described below. We have studied the pulsation and respiration of a motile leaflet of *Desmodium gyrans*, when the cut-end of its stem is dipped in solutions of glucose or its breakdown products, glucose-I-phosphate, pyruvate and of some of the organic acids which form part of the Krebs cycle, like malate, citrate succinate, etc. To some of these substrate solutions different catalysts like thiamin, nicotinic acid and indole acetic acid have been added in very small concentrations. The aim was to find out how far (i) the mechanical pulsations of the leaflet is influenced by the absorption of the different substrate solutions, and (ii) how far the leaflet respiration is thereby affected. It was also a part of our original aim to find out whether in the *Desmodium* leaflet, as in muscles, the energy of mechanical contraction is supplied by the successive breakdown and reformation of energy rich phosphate compounds formed by the interaction between adinosinetriphosphate (ATP) and glucose breakdown products. Owing to the difficulties encountered during the war period of synthesizing ATP in the laboratory, this part of the investigation has been postponed for the present.

§15. *Apparatus for recording simultaneously the pulsation and respiration of Desmodium gyrans.*—The surface area of the leaflet being small—about 1·5 cm. in length and 0·3 cm. in breadth—its respiration is obviously of a very small order. For this purpose a modification of the Microrespirometer described by F. O. Schmitt[17] was used, for measuring the small amount of O_2 consumed during the process of respiration. Two vertical cylindrical glass vessels about 6 cm. in height and 2 cm. in diameter are connected 2 cm. below the neck with a 1 mm. bore glass capillary tube 10 cm. in length. On the sides of each of the glass vessels opposite to the capillary tube connections, a tubular glass vessel is sealed at an angle of 15° below the horizon; the cut-end of the petiole of the experimental leaflet is dipped in one of these glass vessels containing water, so that it can pulsate freely within the vertical glass vessel. A small drop of light apiezon oil is introduced into the capillary tube which separates the air enclosed in the two vessels, in each of which a filter paper soaked in caustic soda is suitably placed for the absorption of CO_2. The ground glass stoppers used for the vertical glass vessels are broad at the base which fit the wide mouths of the vessels and terminate to narrow ground cones with covers at their upper ends. As the leaf respires it takes in O_2 and gives out CO_2, of which the latter is absorbed by the caustic soda soaked in the filter paper; this results in a reduction of pressure inside its containing chamber, giving rise to a movement of the oil meniscus toward that direction.

The object of using similar vessels on both sides is to eliminate the effect of slight variation of temperature on the enclosed air during the period of experiment. Both the vessels will be equally affected by such slight variation of temperature and, therefore, the movement of the meniscus will not be influenced by any small change of temperature. Moreover, the latter is minimized by placing the whole apparatus, fixed on a heavy stand,

within a large glass water bath, so that the pulsation of the leaflet as well as the movement of the oil meniscus can be observed clearly from outside.

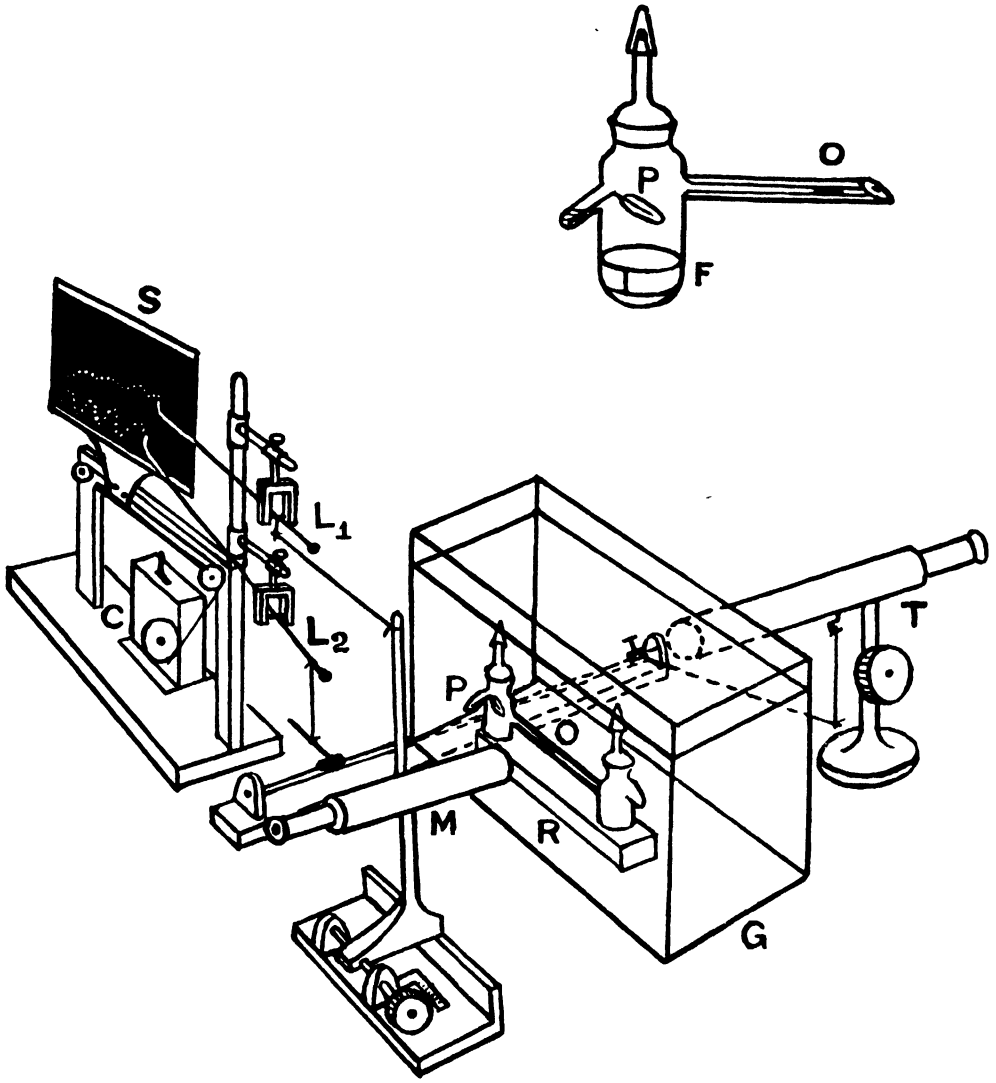


FIG. 7. Apparatus for recording simultaneously the respiration and pulsation of a single *Desmodium* leaflet.

R, respiration chamber placed inside the water bath G; P, plant in the respirometer; O, oil meniscus the movement of which is followed by the travelling microscope M, fitted with micrometer screw; T, micro-telescope fitted with rack and pinion arrangement for following the up and down movement of the leaflet; S. smoked glass plate mechanical recorder worked by the clockwork C; the upper lever L_1, of the recorder is attached with a fine wire to the travelling microscope and thus records the rate of oxygen consumption; the lower lever L_2 is connected with the micro-telescope by suitable cam arrangement and thus records the pulsation of the leaflet. Inset shows the enlarged diagram of one side of the respiration chamber. P, leaflet with its petiole dipped in water in the side tube; F, filter paper soaked with sodium hydroxide placed inside the chamber; O, oil meniscus in the capillary tube.

As the leaflet is enclosed in the glass vessel, recording of its pulsation by direct mechanical means could not be employed. Optical method was found to be suitable for the purpose of recording both the pulsation and respiration. A horizontal travelling microscope fitted with a micrometer screw is placed on one side of the water bath and one observer follows the movement of the oil meniscus in the capillary tube by means of the micrometer screw. The body of the microscope is attached with a fine wire to the upper lever of a smoked plate recorder placed on one side of the microscope, so that the oxygen consumption indicated by the movement of the oil meniscus, is represented by the up-curve in the smoked plate recorder. Another observer follows the up and down movement of the tip of the leaflet with a tele-microscope placed on the opposite side of the chamber and fitted with a vertical rack and pinion arrangement. The movement of the tele-microscope is communicated by suitable cam arrangement to the lower lever of the smoked-plate recorder; thus the up and down movement of the leaflet is represented by up and down movement of the levers in the recorder. The interval between two dots in all the records is fifteen seconds. The complete apparatus is diagrammatically represented in Fig. 7.

In our investigation on respiration we began with taking normal records of respiration of the pulsating leaflet in order to observe whether there is any fluctuation in the rate of respiration concomitantly with the rise and fall of the leaflet. It has been assumed by Bose that the fall of the leaflet is associated with the contraction of the pulvinus. Now if the fall of the leaf is due to contraction, greater amount of energy will be required in the process and under that condition it can be conceived that the rate of respiration will also be correspondingly increased.

EXPERIMENT 4

§16. *Normal respiration of Desmodium leaflet.*—The detached leaflet was allowed to rest for an hour to overcome the shock sustained in cut injury. After that it was mounted in the respiration chamber as described before. The records of pulsation and respiration were taken simultaneously half an hour after the chamber was immersed in water bath for attainment of constant temperature. A typical record of the pulsation and respiration of a leaflet is given in Fig. 8.

From the records given in Fig. 8, it will be noticed that the respiration curve has a periodicity similar to the pulsation of the leaflet. If a straight line is drawn from the beginning to the end of the respiratory curve it is found that the respiration, in spite of periodic rise and fall, maintain a more or less uniform rate. The respiration and pulsation curves were transferred on square paper, in order to see whether the periodicities of the two curves exactly correspond with each other. It was found that the periodic increase or decrease of respiration did not exactly correspond with the up and down movement of the pulsation. There might be a time lag between respiration and pulsation on account of which it is difficult to determine which of the movements—up or down—is exactly associated with the increase or decrease of respiration.

For accurate determination of the volume of oxygen absorbed, the reading of the micrometer screw, which represented the length through which the oil meniscus shifted within the period of one particular experiment, was recorded. The distance travelled by the oil meniscus multiplied by the cross-section of the tube gives the total volume of oxygen absorbed in that particular time. The area of the leaflet was measured by taking its ink impression on a millimeter squared paper and counting the number of squares covered by the impression. The time taken by a particular experiment and the area of the leaf

being known, the volume of oxygen consumed per minute per square millimeter leaf surface can be calculated. The rate of respiration was found to vary from leaf to leaf.

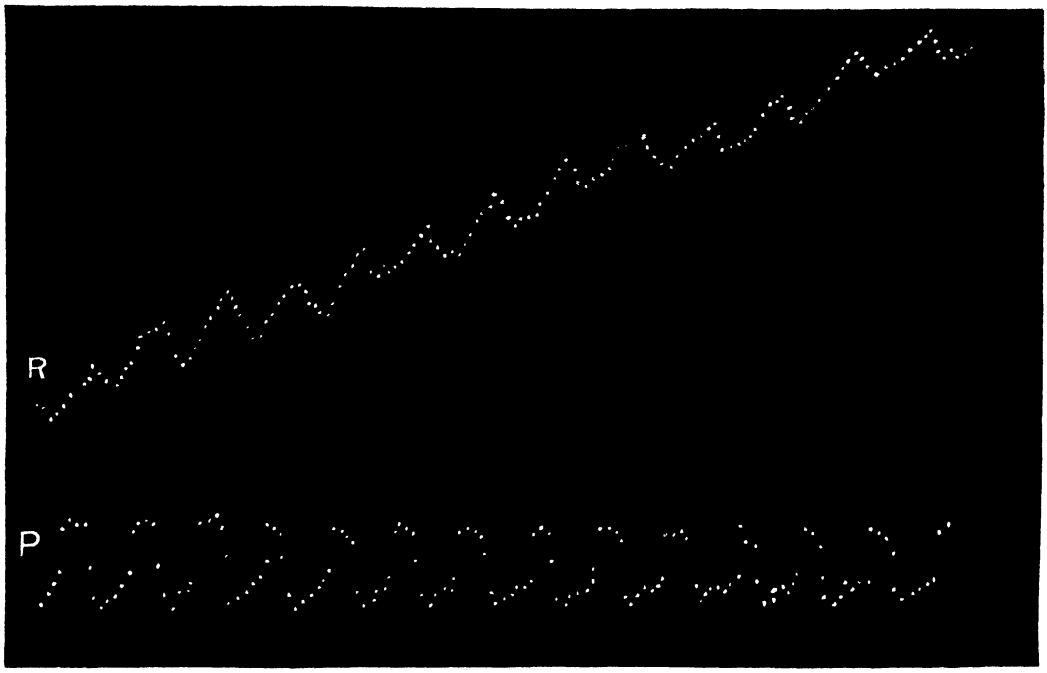


FIG. 8. Normal records of respiration and pulsation of a single *Desmodium* leaflet.
R, rate of respiration; P, pulsation. Note pulsatory periodicities in the rate of respiration similar to pulsation curve.

Therefore, in our present studies of the effects of different substrate solutions, the normal rate of respiration of each leaflet was compared to the change in its rate for the same leaflet, under treatment with different nutrient solutions, and the result is expressed in terms of percentage of increase or decrease.

EXPERIMENT 5

§17. *Respiration in a condition of forced stoppage.*—If the down movement of the leaflet indicates contraction (Bose), greater amount of energy will be required in the process and consequently there will be an increase in the respiration rate. In the up movement of the leaf work against gravity will also require more energy than when the leaf is in a stationary position. Now, taking for granted that contraction induces the down movement, it remains to be seen which of the two reactions—contraction or work against gravity—requires more energy. If a stationary condition is forced upon the leaf its work against gravity will be stopped. But the contractile movement which is outwardly manifested by the fall of the leaf is conceivably initiated in the tissue of the pulvinus. Therefore, in an induced stationary condition, though the down movement of the leaf will be checked the internal metabolism in the pulvinus, due to periodic contraction, will go on and in that condition some more energy will be required resulting in the persistence of the periodicity in the respiration curve. In order to verify this assumption, after taking the records of normal respiration and pulsation of the leaf for a time, the leaf was fixed

to the glass wall of the chamber with a touch of shellac varnish at the tip. The leaf had no visual pulsatory movement under this condition. After taking the respiratory rate in this stationary condition for a certain time, the leaf was set free and again both its pulsation and respiration were recorded. A typical record is given in Fig. 9.

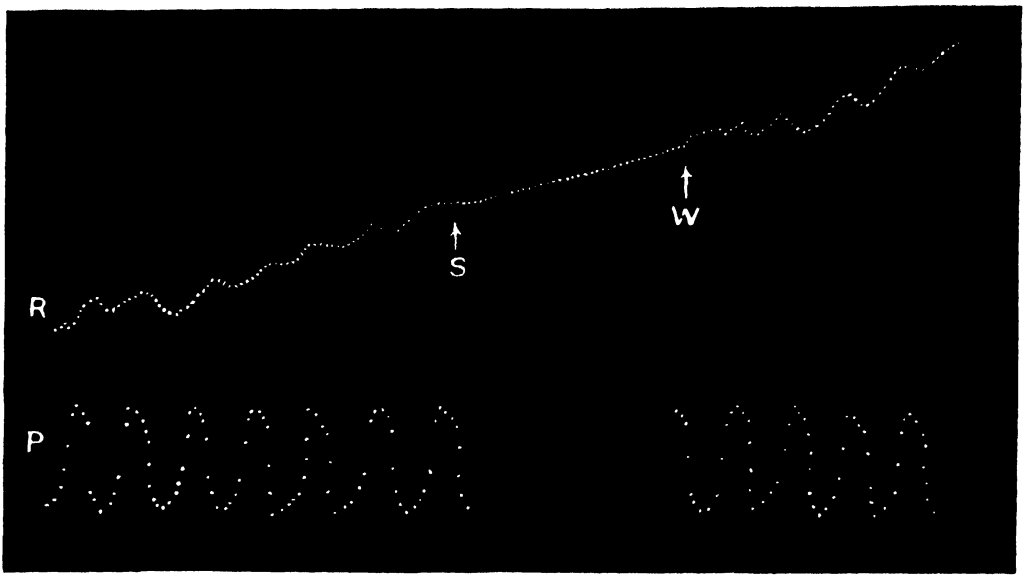


FIG. 9. Records of respiration R, and pulsation P, of *Desmodium* leaflet showing cessation of periodicities in the respiration curve by the forced stoppage of pulsation at S; the periodicities re-appeared after setting the leaflet free at W.

In the record (Fig. 9), the periodicity of respiration is found to disappear completely in a condition of forced stoppage of pulsation. Had the periodic increased respiration been due to the contraction of the pulvinus, the periodicity of respiration would have persisted even after the forced stoppage of the movement of the leaf. Therefore, the periodic increase of respiration may be attributed to the effect of extra amount of work done by the leaf in raising itself against gravity. In down movement the energy requirement in contraction may possibly be minimized by the help of gravity. These results are further considered in the last section under Discussion.

EXPERIMENT 6

§18. *Respiration of an old leaf.*—This experiment was performed only to compare the rate of respiration of new active leaf with that of an old leaf of which the pulsating activity has stopped. A typical record of respiration of an old leaf of which the total area was 26 sq. mm. is given in Fig. 10.

The slow rise of the curve indicates that the rate of respiration was low; the curve is also absolutely devoid of any periodicity. For comparison of the rates of young and old leaves, the rates were calculated per square millimeter per minute as mentioned previously. The rates of a number of leaves as obtained from these records are given in Table I.

It will be found from the values of the respiratory rates in Table I, that the mean rate of respiration of a young leaflet is about four times higher than that of the old leaf.

§19. *Effect of darkness on respiration.*—Light has been found to play an important rôle in the pulsatory activity of *Desmodium.* It has been found from the diurnal record of

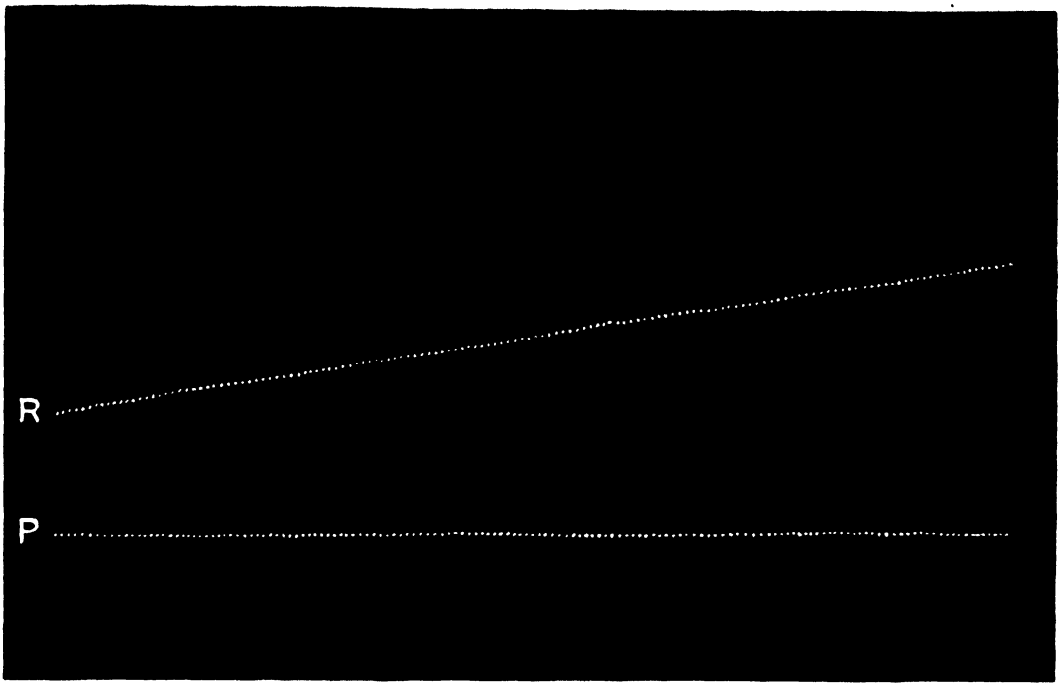


FIG 10. Record showing the rate of respiration R, of an old leaflet of *Desmodium* which had stopped pulsating P. Note disappearance of periodicities in the respiration curve.

TABLE I

Oxygen consumption of single leaflet of Desmodium gyrans

Young (pulsating).		Old (pulsatory activity stopped).	
No. of specimen	O_2 consumption in cu. mm. per sq. mm. per min.	No. of specimen.	O_2 consumption in cu. mm. per sq. mm. per min.
1	0·049	1	0·008
2	0·048	2	0·016
3	0·073	3	0·018
4	0·070	4	0·024
5	0·106	5	0·016
Mean	0·069	Mean	0·016

pulsation that the pulsation gradually diminishes in amplitude and frequency with the nightfall till it stops after a time. It has also been shown from subsequent experiments

that this stoppage of pulsation in the absence of light, is due to the depletion of stored energy accumulated in the presence of light. Now this active leaf, whose stored energy is so rapidly depleted in the absence of light, was used in an experiment to observe how respiration is affected under this condition. Light and darkness have also been found to induce great changes in the different metabolic processes of plant life. Consequently, the energy requirement underlying each of the different metabolic processes may also be conceived to be variable under different environments. Moreover, the requirement of energy due to photosynthesis is nil in darkness. Thus the variable requirement of energy under the different conditions may result in some changes in its liberation through the process of respiration. But most of the workers who have studied the effect of light on respiration have denied that light has any effect on it. Only Van der Paauw [18] reported some data, which show an acceleration of the rate of respiration in light. McAlister [19] and Emerson [20] found in their experiments that the rate of respiration remains the same in light and in darkness and concluded that light has no effect on respiration. According to Briggs [21] some of the elementary products of photosynthesis which, however, yet unknown—preceding the formation of ordinary sugar—may be oxidized more readily than the latter. So according to him there is little ground except convenience, for assuming that respiration in assimilating cells is the same in light as in darkness.

Experiment 7

Effect of darkness on the respiration of Desmodium leaflet.—In observing the effect of darkness on respiration the normal rate of respiration in ordinary light was recorded for a time. After that the vessels on both sides of the respirometer were simultaneously covered by two metallic covers. In a preliminary experiment it was found that when the covers are put on the vessels, sometimes the oil meniscus slightly loses its balance but it does not take more than five minutes to readjust itself. So in recording the respiration in darkness a time gap of fifteen minutes was allowed after covering the respiration chamber, to obviate any error due to mechanical disturbances. The records obtained under darkened condition were compared with the normal to find out if there was any change under the altered condition. A typical record is given in Fig. 11,* for comparison of the rates under the conditions of light and darkness. It will be noticed that the rate in darkness has slightly increased over that of the normal. From a number of experiments it was found that the average increase was approximately 10% over that of the normal. In some of the experiments after taking the record in darkness for a time, the covers were taken out and respiration was recorded in normal light again. The respiration was found to come down to normal rate again. From different records it was also found that the increased rate of respiration slightly declined in continued darkness.

The increase of respiration in darkness is difficult to explain. If it has to be associated with the energy requirement of the plant then the energy requirement of the plant in all its functions taking place in light and darkness will have to be correctly assessed. The effect of light has been observed only in a few of the primary metabolic functions of the plant. In those also a correct estimation of the energy requirement underlying the respective function will not be an easy matter. The other secondary functions will further complicate the matter. If Brigg's assumption is carefully considered it may lead to an explanation in the present case. If the primary metabolites of photosynthesis are consumed in respiration

* In this and in subsequent records the movement of the plate has been reduced by half for prolonged observation.

in presence of light, then comparatively less oxygen will be required to break them, whereas, in darkness when comparatively more complex substances are used up in respiration the oxygen requirement can be assumed to be greater.

FIG. 11. Record showing effect of darkness on the respiration of *Desmodium* leaflet.
R, record of respiration rate; P, record of pulsation. The respiration chamber was darkened at D; the record of pulsation could not be taken after darkening the chamber, which is indicated by the horizontal dots in the pulsation curve.

EXPERIMENT 8

§20. *Effect of glucose on respiration.*—The effect of glucose on respiration was recorded both under light and dark conditions. The experiment was conducted under ordinary room light condition. In experiments on the effect of glucose, the normal pulsation and respiration of the detached leaf placed in ordinary water, was first recorded. After the normal records had been taken for a time, the water was replaced by a freshly prepared glucose solution. In introducing the glucose solution, the respiration chamber had to be taken out of the water bath and opened. So, after the replacement of the chamber in the water bath a considerable time was allowed for readjustment of temperature. The total time required in the operation including the allowance for readjustment of temperature was one hour. Therefore, in the composite respiration curve consisting of both normal and glucose effects, the intervening period was one hour. In order to produce a continuity of the curve, for convenience of comparison, the recording levers of the respiration and pulsation recorders were brought to the ends of the normal curves so as to make the two contiguous. A typical record is given in Fig. 12.

In the above record the respiratory rate in glucose was found to increase by 172% over that of the normal. The periodicity of normal respiration curve was almost absent

when the rate was accelerated by the application of glucose solution; this may be attributed to the high rate of respiration which made the periodicity inconspicuous. A number of

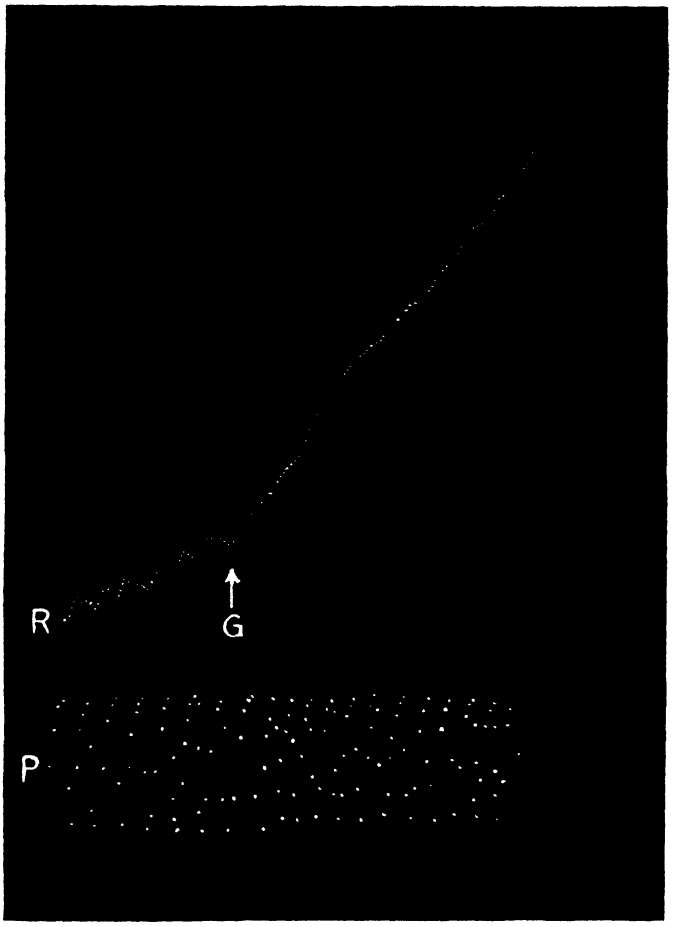


FIG. 12. Record showing effect of glucose on the respiration and pulsation of *Desmodium* leaflet. R, rate of respiration; P, record of pulsation. Note considerable increase in the rate of respiration after application of 1% glucose at G.

experiments were performed with 1% glucose and in all cases there was increase in the respiratory rate varying from 250 to 350%. Application of glucose also slightly increased the frequency of pulsation, which was determined by counting the number of dots of individual pulse under glucose treatment with that of the normal. The average of several records showed only 4% increase on treatment with glucose. The amplitude variation was not noticeable (see Table II).

Glucose in lower dilutions was also tried. The minimum concentration effective was found to be 0·25%, below which the normal rate remained unchanged. The rate of increase was, however, correspondingly lowered along with the gradual lowering of the percentage of glucose below 1%.

Effect of Thiamin and Nicotinic Acid.—The enzyme carboxylase can decarboxylate Pyruvic acid→Acetaldehyde + Carbon dioxide. This enzyme, which has been isolated in

a crystalline form, has been found to contain a pyrophosphate of thiamin. L. E. Hawkes [22] has found an increase of glucose consumption by Melanspore, when to the liquid nutrient media thiamin is added.

Similarly nicotinic acid amide is a constituent of both coenzyme I and coenzyme II. The former functions as coenzyme for many dehydrogenases. Its action consists in taking up of two hydrogen atoms, whereby dihydro coenzyme I is formed. Coenzyme II also acts in enzymic reactions by taking up two atoms of hydrogen. Its pyridine residue (nicotinic acid amide) gives the compound this property. These chemicals being easily available, it was thought worth while to find out whether their addition in very small concentrations affected the respiratory activity of *Desmodium* leaflets.

Thiamin and nicotinic acid, both in 0·001% concentration were individually added with 0·05% glucose, at which concentration of glucose no change in the respiration of the leaflets had been observed. Addition of these substances was found to induce no change in the respiratory activity.

Experiment 9

§21. *Effect of Glucose in darkness.*—In this experiment the normal rate of respiration was taken in darkness. The leaf was kept in darkness for an hour before the commencement of recording. After the normal rate was recorded for a time, glucose was added and in doing so the chamber had to be opened as stated before and an hour elapsed before the next recording commenced. The increase over normal was not much different from that obtained under lighted condition.

The mean values of the effects of different concentrations of glucose on respiration and pulsation, both in light and darkness, and also the joint effects of thiamin and nicotinic acid with glucose, are given in Table II. The normal rates of respiration and pulsation in each curve have been taken to be 100, and the rates representing the effects of respective solutions have been compared to the normal on percentage basis. Both the amplitude and frequency have been taken into consideration in observing the effects on pulsation.

Table II

Effects of various concentrations of glucose on the respiration and pulsation of Desmodium gyrans

Treatment.	Respiration ratio between normal and treated.	Pulsation.	
		Frequency ratio between normal and treated.	Amplitude ratio between normal and treated.
Glucose 1%	100 : 293±15·9	100 : 104·3±4·2	100 : 100
Glucose 1% in darkness ..	100 : 280·8±19·2	....	....
Glucose 0·5%	100 : 191·9±8·6	100 : 107·5±5·5	100 : 95±5
Glucose 0·25%	100 : 133·4±13	100 : 101·6±3·6	100 : 103·3±3·8
Glucose 0·05%	100 : 108·3±8	100 : 101·1±5	100 : 100
Glucose 0·05% + Thiamin 0·001%	100 : 98·5±6·5	100 : 98±2	100 : 95±5
Glucose 0·05% + Nicotinic acid 0·001%	100 : 101·1±7·8	100 : 97·5±1	100 : 100

It will be found from the Table II that 1% glucose has induced about 200% increase in respiration rate. In darkness also the increase in respiration due to glucose is almost the same. The acceleration in the respiration rate is seen to be progressively lowered with the lowering of the percentage of glucose, the lowest being at 0·05%, which may be considered as almost normal. Joint application of thiamin or nicotinic acid with glucose 0·05% did not produce any appreciable change from the normal. With 1 and 0·5% glucose the frequency of pulsation is seen to be slightly increased. The small variations in amplitude in some can be neglected, as it may occur even in normal records.

§22. *Effect of Glucose-1-Phosphate on Respiration of Desmodium leaflet.*—We shall now consider the effect of supplying the *Desmodium* leaflet with carbohydrate breakdown products. These carbohydrates occur in plant tissues as starch, fructose and glucose, which during the process of phosphorylation give rise to a common breakdown product, fructose-6-phosphate, after which they all undergo identical degradation process. The step reactions followed by the three starting materials can be represented as follows:—

$$\text{Starch} + H_3PO_4 \xrightarrow[\text{(phosphorylase)}]{} \text{Glucose-1-phosphate} \rightarrow \text{Glucose-6-phosphate} \rightarrow \text{Fructose-6-phosphate}$$

$$\text{Glucose} + \text{ATP} \xrightarrow[\text{(hexokinase)}]{} \text{Glucose-6-phosphate} \longrightarrow \text{Fructose-6-phosphate}$$

$$\text{Fructose} + \text{ATP} \xrightarrow[\text{(hexokinase)}]{} \text{Fructose-6-phosphate}$$

Of all these breakdown products, it was possible only to prepare in a pure form Glucose-1-phosphate in our Chemical Department, and so we investigated the effect of putting the cut-end of the stem of a pulsating *Desmodium* leaflet in different concentrations of solution of this substance.

EXPERIMENT 10

Glucose-1-phosphate was prepared in the Chemical laboratory of the Institute from potato starch. Freshly prepared glucose-1-phosphate was used in different concentrations ranging from 0·01 to 1%. None of the concentrations used produced any change in the normal rate of respiration and pulsation within the time limit of the recording. When the cut-end of the specimens were dipped in 1% or 0·05% solutions for about twenty-four hours their rates of respiration were found to be greatly diminished and the pulsating activity completely stopped. Some of the specimens were kept in different concentrations of solutions for visual observation of their effect on pulsation. The specimens kept in solutions from 0·02 to 1% lost their power of pulsation within twenty-four hours and the leaves withered within three to five days. In 0·01%, however, the pulsating activity was retained up to three to four days. It is not easy to interpret the negative result obtained, as glucose-1-phosphate is not an immediate breakdown product either of glucose or fructose. The latter is a degradation product of sucrose, which is the form in which stored sugar is found in plant cells. It is necessary to find out the different forms in which carbohydrate is stored in *Desmodium* leaflets.

§23. *Effect of pyruvic acid on the respiration of Desmodium leaflet.*—We have seen that pyruvic acid represents the last stage in the breakdown of all phosphorylated carbohydrate compounds, resulting in the formation of trioses. It is generally accepted that pyruvic acid is the starting point for those breakdown processes in animal tissues which

lead to the production of CO_2. Sixteen different reactions have been found in which pyruvic acid can take part. We have, therefore, considered it desirable to investigate the effect on respiration, using a pyruvate solution as a nutrient, on the expectation that it will be readily utilized for respiration and thereby increase the rate of oxygen absorption.

EXPERIMENT 11

Sodium pyruvate was prepared from pyruvic acid in the Chemical laboratory of the Institute. Different concentrations ranging from 0·1 to 1% were applied, but the rate of oxygen absorption was not accelerated at any of the concentrations within the time limit of our observation. There was no change in the pulsation rate as well. When the specimens were kept in the solutions, for visual observation of their effects on the pulsatory activity, they behaved similarly to those kept in glucose-1-phosphate solutions. Pulsations of the leaflets stopped on the following day and the leaflets, kept in 0·5 and 1% solutions, dried up within two to three days, showing that either the substance itself has produced a toxic effect or the normal suction of the leaf was obstructed in some way by the application.

§24. *Effect of C_4 acids on the respiration of Desmodium leaflet.*—The C_4 acids have been tried by different workers on isolated animal tissues and there is ample evidence that they function catalytically in the biological oxidation and that small amounts of these acids can bring about an increase in respiration not by being themselves oxidized but by stimulating the oxidation of some other substrate in the tissue. There is a suggestion, however, that when present in sufficient quantity C_4 acids are themselves used up in respiration. As stated in Introduction the C_4 acids participate in both the Szent Gyorgi and the Krebs cycle.

EXPERIMENT 12

In our present investigation on the effects of C_4 acids, salts of malic and succinic acids were used in different concentrations. Effect of citric acid which contains a greater number of carbon atoms and is included in the Krebs cycle, was also investigated with the C_4 acids. Malate, succinate and citrate in 0·01% solutions were absolutely ineffective in producing any change in respiration. In 0·05% solutions also succinate and citrate were almost ineffective but malate induced slight diminution both in the rate of respiration as well as amplitude of pulsation. With 1% solution marked decrease in the rate of respiration and pulsation was found to occur in all. No acceleration in the rate of respiration was found to occur in any of the specimens treated with the varying concentrations of the above salts. Prolonged treatment with these salts was found to induce a toxic effect on the leaflet. In 1% and 0·05% solutions the pulsation was found to be stopped on the very next day. In 0·01% solution the pulsation continued feebly for two and in some cases even up to three days.

§25. *Auxin as Respiratory Catalyst or Co-enzyme.*—The effect of auxin on the growth reaction of the plant seems to induce a master reaction, the fundamental nature of which is yet unknown. But the linear proportionality, as obtained by different workers, between the quantity of auxin applied and growth indicates that auxin enters into definite stoichiometric reaction with some constituents of the cells. Since a close connection exists between growth and respiration, this consideration led many workers to investigate the effect of auxin on respiration, so that some light may be thrown on the fundamental reaction from this direction.

Effect of auxin has been studied on the respiration of *Avena coleoptile* by some workers, but the observations of the different workers are not concordant. Bonner [29] and Hulssen [30]

could not find any increase of respiration in sections of *Avena coleoptile* by treatment with auxin, but the results of Sweeney and Thimann [24] show that auxin accelerates the rate of respiration of the *coleoptile*. Later Thimann and Commoner [24a] showed that *Avena coleoptile* previously soaked in malate produced marked increase in respiration upon addition of auxin. From this and from other evidences they concluded that auxin must play the part of a catalyst or co-enzyme of the four carbon acids in the respiration cycle postulated by Szent Gyorgi. According to Avery [25] the increase in respiration reported by the investigators is very small and better proof must be found before any generalization on the effect of auxin on respiration can be made. We decided to test the validity of the observations of the previous authors on the respiration of *Desmodium* leaflets.

First of all we recorded separately the effects of auxin and malate on respiration. Then we tested the joint effect of auxin and malate, and compared it with their individual effects on respiration. We did not use sucrose or glucose as substrates, as Thimann and Commoner had done in their experiments on *coleoptile tip*. The effect of glucose was so highly accelerating on the respiration of *Desmodium* leaflet that the effect of any other substance applied in combination with it would be completely masked by it. Moreover, as the experiment was completed within a very short time, the carbohydrate reserve in the tissue may be assumed to be in sufficient quantity for the catalytic reaction if any. The maintenance of pulsatory activity also substantiate this assumption.

Experiment 13

Effect of auxin and auxin-malate on respiration of Desmodium leaflet.—Indole acetic acid and sodium malate were used in 0·0005 and 0·05% solutions respectively. In each experiment normal records were taken for a time in tap water; after that the water was replaced by one of the solutions and the recording was resumed after an hour. Therefore, the leaflet was treated with the respective solutions for an hour before its effect was recorded. In studying the joint effect of auxin-malate the solution used contained 0·05% malate and 0·0005% of indole acetic acid. Typical records of individual and joint effects of indole acetic acid and sodium malate are given in Fig. 13. The summarized results of different experiments are given in Table III. The results are expressed on percentage basis compared to those of the normal.

Table III

Effects of individual and joint applications of indole acetic acid and sodium malate on the respiration of Desmodium leaflet

Treatment.	Respiration ratio between normal and treated.	Pulsation.	
		Frequency ratio between normal and treated.	Amplitude ratio between normal and treated.
Sodium malate 0·05% ..	100 : 91·29 ± 4·6	100 : 96·76 ± 3·04	100 : 84·5 ± 5·6
Indole acetic acid 0·0005% ..	100 : 113·86 ± 9·65	100 : 95·81 ± 2·1	100 : 85·8 ± 8·03
Sodium malate 0·05% and Indole acetic acid 0·0005%.	100 : 152·19 ± 10·03	100 : 92·05 ± 1·6	100 : 99 ± 8·8

The Table III shows that 0·05% sodium malate induces a diminution in the rate of respiration as well as in the amplitude of pulsation. With 0·0005% indole acetic acid

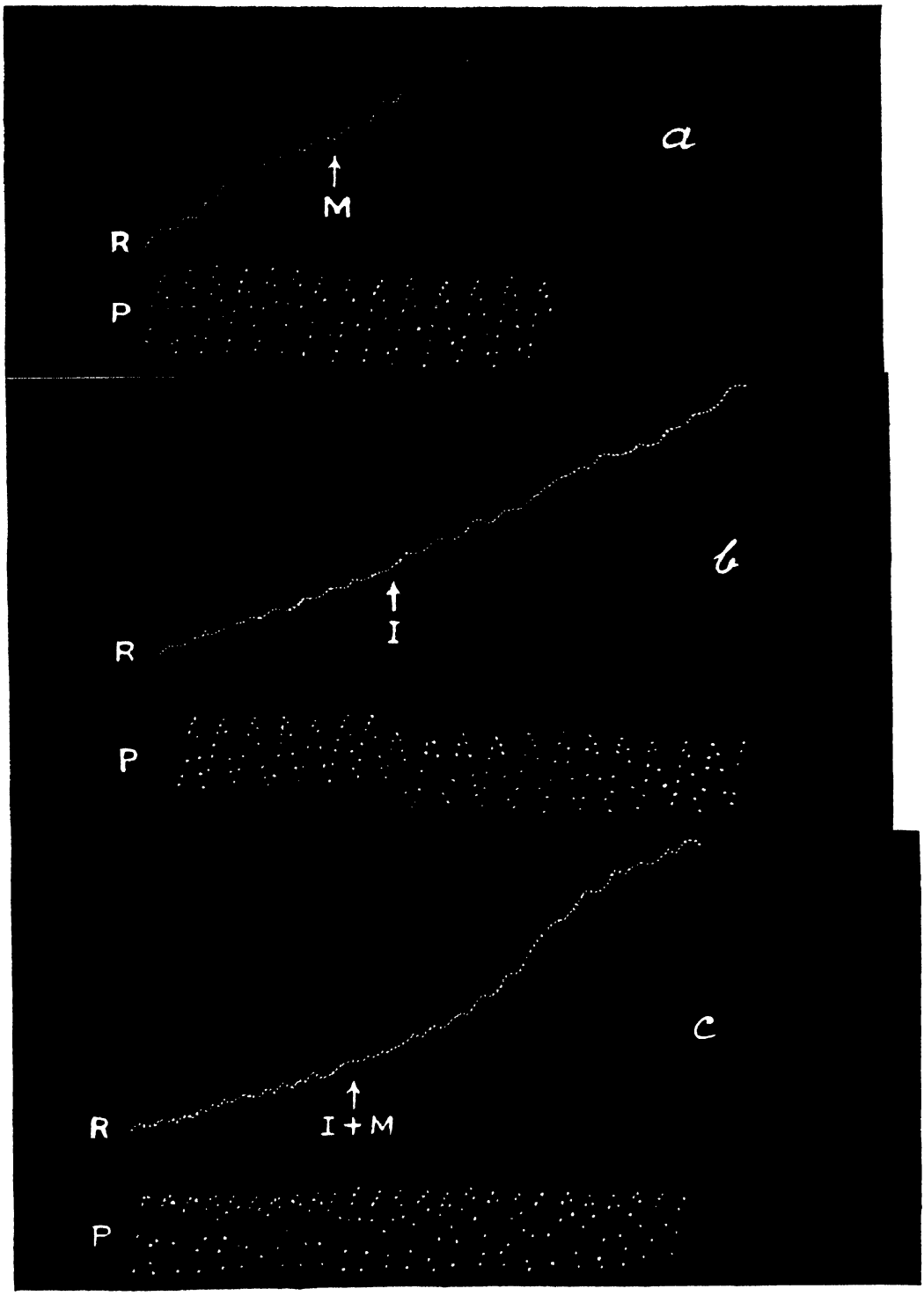


FIG. 13. Records of individual and joint effects of sodium malate and indole acetic acid on respiration R, and pulsation P, of *Desmodium* leaflet:—
(*a*) application of 0·05% sodium malate at M;
(*b*) application of 0·0005% indole acetic acid at I;
(*c*) application of 0.0005% indole acetic acid with 0·05% sodium malate at I + M.

there is some increase in the rate of respiration and diminution in the rate of pulsation. When treated with a solution containing 0·05% malate and 0·0005% indole acetic acid, a 50% increase in the rate of respiration is observed, with a slight reduction of the frequency of pulsation.

In order to observe whether indole acetic acid induces the same acceleratory effect on respiration in combination with any other substrate, 0·0005% of the former was applied in combination with 0·05% sodium succinate and 0·05% sodium citrate; but no acceleration was found to occur similar to its combined effect with malate. Again, 0·0005% indole butyric acid was applied in combination with 0·05% malate, but this also induced no acceleratory effect on respiration. This led us to conclude that indole acetic acid is a co-enzyme or catalyst for malate in its utilization in the respiration of *Desmodium gyrans*.

Discussion

§26. We have found in the course of the investigations recorded in Part II that accompanying the mechanical pulsations of the *Desmodium* leaflet, there is a periodicity in the rate of respiration in the leaflet of approximately the same period as the mechanical pulsation. There is a certain amount of phase shift between the two sets of periodic curves, which makes it difficult to correlate the different portions of the up and down movements of the leaflet with those of the respiration curve. The latter curve can be looked upon as made up of a straight line inclined at a definite angle with the time axis, which represents the respiration due to the general metabolism of the leaflet, and superposed on it is the periodic curve which represents the variation in the rate of respiration due to the mechanical work done by leaflet during its rise against gravity and on the leaflet by gravity during its downward course. It is interesting to note that Bose (*Comparative Electrophysiology*, p. 277) had recorded the electric pulsation accompanying the mechanical pulsation of a Desmodium leaflet by connecting the terminals of a sensitive galvanometer, one to the pulvinated joint and the other with a common petiole. It was found that corresponding to each mechanical pulsation there is a double electric pulsation—a large principal followed by a smaller secondary wave. On recording simultaneously both the pulsations on a photographic plate, it was found that the subsidiary wave of relatively small amplitude and large period corresponded to the slow up movement of the leaflet, and the principal wave characterized by large amplitude and small period coincided with the quick down movement of the leaflet.* The galvanometer deflections indicated a condition of galvanometric negativity of the pulvinule at the moment of its up and downward movement. Even when the leaf is mechanically constrained from executing any movement, the electric pulsation was found to persist with even greater vigour. It appears as if the fundamental excitatory reaction being deprived of one of its mode of expression, exhibited the other with greater energy. According to Bose electrical and mechanical responses are independent modes of expression of the single fundamental process of excitation in *Desmodium* leaflet.

Contrary to the non-abolition of electric excitation under mechanical constraint, we have found that the periodicity in respiration disappears under such condition. One

* Guha-Thakurta and Dutt (*Trans. Bose Res. Inst.*, XV, 157, 1942-43) have shown that the actual movement of the *Desmodium* leaflet is elliptical, with continuously changing shape, from a straight line to a circle. When the movement is pronouncedly elliptical, its vertical component as recorded by crescograph has a diphasic character, similar to that shown by the electrical pulsation. Such a pair of diphasic mechanical and electrical pulsations is shown in Bose's *Motor Mechanism in Plants* (p. 327).

possible explanation of the discrepancy is that the periodicity in respiration is an artifact, due to air currents produced in the chambers by the movement of the *Desmodium* leaflet.* Against this may be urged the observation recorded in Experiment 8 that the periodicity of normal respiration was almost absent, when the latter was accelerated by glucose solution. In this case the amplitude of mechanical pulsations was the same as in water and there was a certain rise in frequency. If there is any relation between the electric potential developed at the pulvinule with capacity for performance of mechanical work by the attached leaflet, then work is being done by the latter both during the up and down movement. The periodicity shown in the respiration curves represents then the difference in the work done during the up and down movements. It appears as if this difference is abolished when the leaflet is mechanically constrained to remain still. The absence of periodicity under such condition may also be due to the fact, that the method used to determine the oxygen consumption in the respirometer by the motion of an oil drop in the connecting capillary tube is subject to large viscous resistance, and as such the arrangement is not capable of recording the finer variations in the respiration of a mechanically constrained leaflet.

In the discussion at the end of Part I of this paper, attention was drawn to Rashevsky's theory of coupled reaction, which may be used to interpret the mechanical pulsations in the *Desmodium* leaflet, as being conditioned by the periodicity in the chemical reactions responsible for the supply of energy. This theory would be able to explain the persistence of electric pulsations in the leaflet pulvinule, in the absence of mechanical movement in the leaflet.

Respiration of a young leaf is seen to be approximately four times greater than that of an old one in which the pulsatory activity has stopped. The respiration curve of the old leaf, though with a low slope is uniform and devoid of any periodicity like that of the active young leaf. The slow respiration rate of the old leaf indicates that metabolism is slower and the uniformity of the curve is due to its loss of pulsatory activity.

In darkness the respiration rate has been found to increase considerably. It is difficult to give a correct explanation to it. Most of the workers have found that light has no effect on respiration and only Van der Paauw [18] found a difference. He found an increase in light and not in darkness and so his finding is quite opposed to ours. In darkness the energy source becomes reduced due to the absence of photosynthesis. It can, therefore, be reasonably conceived that if there is any increase, it ought to be in light and not in darkness. But if Brigg's [21] assumption has any value a suitable explanation can be given to our result: If the primary metabolites of photosynthesis are consumed in respiration in presence of light, then comparatively less oxygen will be required to break them, whereas, in darkness when more complex substances are used up in respiration the oxygen requirement can be assumed to be greater.

Glucose in 1% concentration has been found to increase the rate of respiration by about 200%, both in light and in darkness. In the highly accelerating rate of respiration

* To test this point the following experiment was undertaken :—

A model paper-leaflet, approximately of the size of a natural one, attached with a piece of iron wire representing the petiole, was introduced in the respiration chamber and the apparatus was set up as in the actual experiment. The movement of the model leaflet with the petiole was induced by intermittent excitation of an electromagnet, placed over the iron petiole outside the side tube of the respiration chamber. The oil meniscus, observed through the travelling microscope, was found to remain stationary in spite of the movement of the model leaflet. Therefore, it is concluded that the respiratory pulsations were due to changes in the respiration and not due to air draught as suspected above.

due to the effect of glucose the periodicity of normal respiration is seen to have become inconspicuous. When the concentration of glucose was lowered below 1% the acceleration due to the effect of glucose also decreased progressively. It is known that glucose before being finally respired into CO_2 and water, has to undergo various dissimilation in the tissue by the action of different enzymes. In the present experiment the respiratory rate is found to be progressively increased with increasing concentration of glucose. This indicates that the respiratory enzymes are present in sufficient concentration in the tissue of *Desmodium gyrans*, to deal with additional quantities of glucose. Therefore, *Desmodium gyrans* is seen to be auxo-autotropic so far as the respiratory enzymes are concerned.

Glucose-1-phosphate and pyruvate the two dissimilated products of glucose under glycolysis, instead of increasing the rate of respiration in any way, produced an inhibiting effect in higher concentrations. From the continued effect on the plant it becomes evident that they induce a toxic reaction on the plant. It seems strange that the effect of any of the dissimilated products will react so differently from the mother substrate. From comparison of the result of glucose with those of its breakdown products it may seem plausible that there may exist in the tissue of *Desmodium* some oxidase enzymes which can affect direct cleavage of carbohydrate without zymase cleavage like those observed by Lundsgaard [23] in yeast, and mould fungi.

We have found that salts of C_4 acids like malate and succinate, as well as citrate in low concentration, do not in any way influence the respiration of *Desmodium* leaflets, while at higher concentrations they produce diminution both in the rates of pulsation as well as of respiration. Prolonged treatment with these solutions produce toxic effect on the leaflets.

On the other hand, low concentration of indole acetic acid produce a slight rise in the rate of respiration, while a combination of 0·05% of malate and of ·0005% of indole acetic acid causes a 50% rise in the rate of respiration. But no change in respiration is produced when (i) the malate is replaced by succinate, and (ii) when to the malate solution indole butyric acid is added, in place of indole acetic acid. This indicates that the latter is a respiratory catalyst for malate as substrate; this is in agreement with the findings of Thimann and Sweeney.

Recently a similar series of investigations was carried out by Bennet Clark and Benson [26] (to be referred to later as B.C. and B.), on the respiration of slices of beet-root. It was found that the rate increased considerably when the water, in which the slices were placed, was replaced by the expressed sap of the beet root. Further investigations showed that organic acids, particularly malic and citric acids, were the sap constituents responsible for the observed effect; this was confirmed by experiments in which the beet root slices were placed in solutions of sodium salts of malic, succinic and citric acids. On comparing the loss of the organic salt molecules with the CO_2 molecules evolved from the slices immersed in these solutions, in excess of the amount given out by the same tissues respiring in ordinary water, it was found that about one molecule of acid was lost for every molecule of CO_2 evolved. The following reaction has been proposed by them to account for the observed effect in case of malic acid.

$$\underset{\text{malic acid}}{C_4H_6O_5} \longrightarrow \underset{\text{hexose}}{\tfrac{1}{2}C_6H_{12}O_6} + CO_2$$

i.e. hexose is synthesized from a decarboxylation of malic acid. Support for the view that malic acid is not merely oxidized to CO_2 and H_2O is provided from measurements on the R.Q. (respiratory quotient) in the tissue supplied with malate. The value is found to

vary between 1·5 and 2·3, and is higher than the one provided by the complete oxidation of malate (1·33). It would, however, be provided by decarboxylation of a molecule of malic acid leading to the formation of one molecule of CO_2 and half a molecule of hexose.

Certain investigations by Sir J. C. Bose and published in his book, *Physiology of Photosynthesis*, appear to support the conclusion, that under certain conditions malic acid can synthesize hexose with the evolution of CO_2. Bose describes some experiments in which the volume of oxygen evolved when the aquatic plant *Hydrilla*, immersed in water charged with CO_2, is illuminated with light of different intensities and wavelengths. If instead of CO_2 the water contains concentrations of malic acid of the order of 0·01%, then O_2 is again evolved under illumination of the immersed plant. It is shown that under constant illumination, in solutions containing CO_2 resp. malic acid in different concentrations, there is a remarkable similarity in the amounts of O_2 evolved when there is proportional changes in the concentrations of the resp. solutes. Bose interprets his observations in a way similar to that proposed by B.C. and B., viz. the first step in the reaction is a synthesis of hexose with a release of CO_2; the latter is utilized for the purpose of photosynthesis by the aquatic plant. Reviewing the results of our investigations in the light of the interpretation given by B.C. and B., of theirs and which is supported by the observations of Bose, we arrive at the following tentative conclusions.

The increase in respiration observed in *Desmodium* leaflets when supplied with a solution containing malic and indole acetic acid in small concentrations is probably not due: (*a*) either to the occurrence of Szent Gyorgi cycle—since the respiration would have shown similar enhancement when the malate is replaced by a succinate, (*b*) or to the occurrence of a Kreb's cycle—since the respiration would have shown similar enhancement, when the malate is replaced by a citrate or a pyruvate.

It seems likely that the mechanism proposed by Bennet Clark and Benson, of the synthesis of hexose from malic acid with the evolution of CO_2 can account for the observed effect. This conclusion will be better established when on measurement, the respiratory quotient of the reaction is found to be significantly greater than 1·33. In view of the extremely minute quantities of material involved in the respiration of a *Desmodium* leaflet, it will be difficult to measure the R.Q. In the absence of such confirmatory test the conclusion (*a*) resp., (*b*) cannot be definitely ruled out. Even in the case of malic acid, enhancement of respiration occurs only when indole acetic acid in small concentrations is added to the nutrient solution. It can happen that when proper catalysts are discovered, similar enhancement of respiration would occur with nutrient solutions containing either citrate or succinate. On this view the negative results obtained with the last two nutrient solutions may be due to the fact that either or both of the two cycles can and do occur in *Desmodium*, but due to the inadequacy of the necessary catalysts or co-enzymes in the leaflet, it cannot utilize any additional amounts of these two nutrients supplied to it. This view, however, appears to be not compatible with (i) our other observation that the leaflet is able to imbibe glucose from nutrient solutions and use it either for increased respiration or for purpose of storage. There is progressive increase in the rate of respiration with increased concentration of glucose, so that with 1% glucose solution the respiration is increased by over 200%. In all known schemes of glucose breakdown in plants, pyruvic acid is taken to be one of the necessary intermediate products. Here again a measurement of the R.Q. in *Desmodium* leaflet, may supply some information as to the ultimate fate of the glucose imbibed. (ii) Thiamin pyrophosphate is a co-enzyme for the decarboxylation of pyruvic acid, but the rate of respiration of a *Desmodium* leaflet in a weak glucose solution was not enhanced by the addition of

a low concentration of Thiamin. Further investigations for the elucidation of some of the problems which have arisen in course of the investigations reported above are being undertaken.

SUMMARY

The investigation was undertaken with a view to understand the energy mechanism of the pulsating activity of the *Desmodium* leaflet; the exact form of energy which is utilized in pulsation, its differential dissipation, if any, in the systole and diastole of the movement and any other accessory mechanism that may be linked up with the energy mechanism.

A photo-recording device has been described for automatically recording the diurnal variation of the pulsatory activity of *Desmodium gyrans*.

An automatic mechanical recorder has been constructed for recording the diurnal variations of light and temperature in the experimental chamber.

The young leaf, under detached condition, stops pulsating after nightfall and resumes again at daybreak; such a leaf lives and retains its pulsatory activity for about twenty days. The medium aged leaf under the same condition goes on pulsating continuously throughout day and night, but comes to a stop within two to three days. The old leaf which is sluggish in activity, shows irregular pulsations throughout day and night; the pulsation hardly survives beyond twenty-four hours.

The stoppage of pulsation of the young leaflet at night has been found to be due to the absence of light and not due to the reduction of temperature at night. The slight reduction in the frequency of pulsation at night has been ascribed to the lower temperature.

In the young leaf supplied with 1 % glucose solution, the nocturnal stoppage disappears and the leaf pulsates day and night under the condition. From this it was concluded that glucose can be a substitute for the photosynthetically stored energy. After the withdrawal of glucose its effect persisted for as long as four days; this indicates that glucose can be retained in the tissue in some form or other for utilization in the plant.

By the application of glucose in complete darkness the pulsatory activity of a young leaf continued day and night in a comparatively feeble manner till the second day of its treatment; after that the pulsation stopped and it was not resumed when brought back to normal light. This shows that though glucose can supply the energy requirement of the plant, it cannot totally replace light in the maintenance of the vital activity of the plant.

For studying the respiration of a single leaflet, a micro-respirometer was devised for measuring the absorption of O_2; the O_2 consumption and pulsation of the leaflet were simultaneously recorded in a mechanical recorder.

The normal respiration curve of the pulsating leaf showed a periodic increase and decrease in the rate of oxygen consumption, indicating a differential dissipation of energy with the rise and fall of the leaf. The disappearance of such periodicity under fixed condition of the leaf appears to support this conclusion. The periodic increase and decrease of oxygen consumption in the respiration curve, could not be directly correlated with the rise and fall of the pulsating leaf. Attention is drawn to a previous investigation of Sir J. C. Bose on the double periodicity of electric pulsations which accompany the mechanical ones in *Desmodium* leaflet, and which persists even when the latter is abolished by mechanical constrants. The relations between mechanical, electrical and respiratory pulsations are discussed in the light of Rashevsky's model of coupled reactions underlying metabolic breakdown of glucose in living cells and which under certain conditions can be of a periodic character.

The respiration of the young leaf per sq. mm. per min. is four times greater than that of the leaf of which the pulsatory activity has stopped. There is no periodicity in the respiration curve of the old leaf.

The rate of respiration was found to increase by 10% under darkened condition.

The rate of respiration was increased by about 200% by treatment with 1% glucose both in light and darkness. The frequency of pulsation was also slightly increased under glucose treatment.

Treatment with glucose-1-phosphate and sodium pyruvate, the two dissimilated products of glucose under glycolysis, did not produce any acceleratory effect on respiration. As a result of continued treatment they were found to induce a toxic effect on the plant.

Succinate, malate and citrate, the components of Szent Gyorgi's and Kreb's cycles, were found to induce no acceleratory effect on the respiration. Continued application of these salts also induced a toxic effect on the plant.

Treatment with 0·0005% indole acetic acid increased the rate of respiration by about 13%. Again, a treatment with 0·0005% indole acetic acid in combination with 0·05% malate increased the rate of respiration by about 52%. Indole acetic acid of the same concentration did not produce any acceleratory effect on respiration in combination with succinate or citrate. Neither indole butyric acid individually nor in combination with malate induced any acceleratory effect on respiration. From these experiments it is concluded that indole acetic acid is a respiratory co-enzyme or catalyst for malate utilization in *Desmodium gyrans*.

The experimental results described in the two parts of the paper, are discussed in the light of recent theories of photosynthesis and breakdown of carbohydrates in plants.

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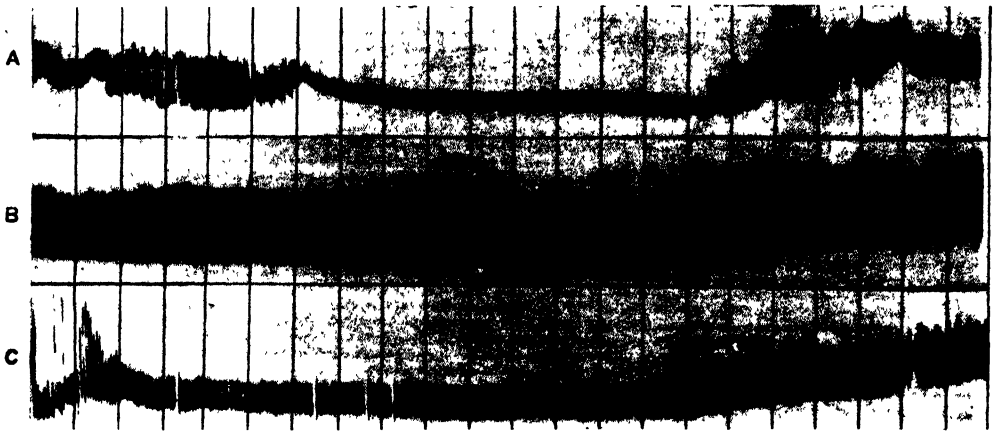

FIG. 4. Records of pulsatory activity of *Desmodium* leaflet under the stages A young, B medium, and C old. The records were started at 1 p.m.; interval between two vertical lines represents one hour.

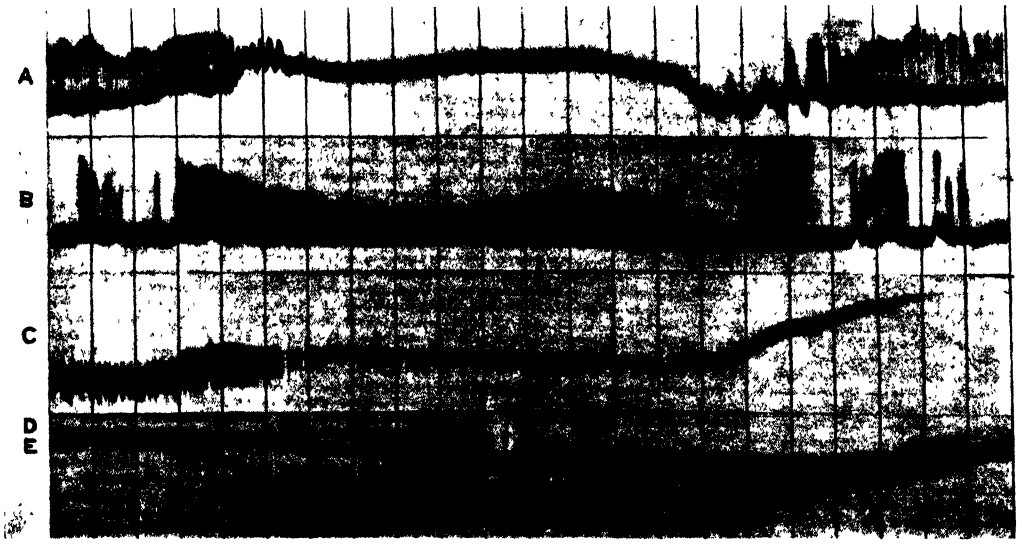

FIG. 6. Records showing the effect of glucose on the pulsatory activity of *Desmodium* leaflet in darkness. A, normal record; B, effect of glucose in darkness on the first day; C, effect of glucose in darkness continued on the second day; D and E, effect of bringing the plant in light and withdrawal of glucose. The records were started at 1 p.m.; interval between two vertical lines represents one hour.

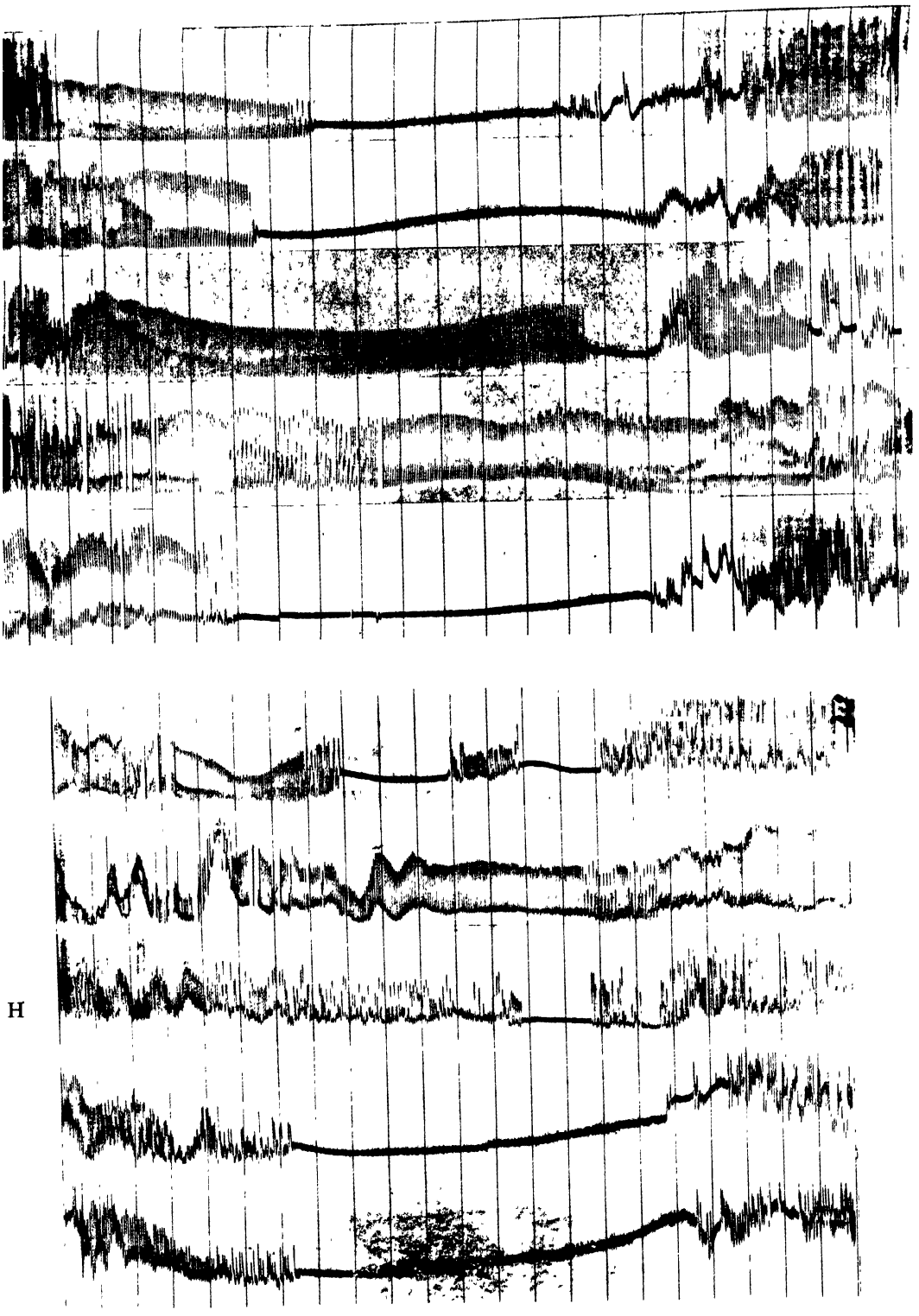

FIG. 5. Records showing the effect of glucose on the pulsatory activity of *Desmodium* leaflet.

XIII. ON THE CHEMICAL NATURE OF THE SUBSTANCES WHICH ARE (I) EFFECTIVE IN THE TRANSMISSION OF EXCITATION IN *MIMOSA PUDICA*, AND (II) 'ACTIVE' IN THE CONTRACTION OF ITS PULVINUS

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INTRODUCTION

§1. Some of the important contributions of Sir J. C. Bose (1906–1926) to Plant Physiology deal with investigations on the relation between stimulus and response in motile plants like *Mimosa pudica*, *Biophytum sensitivum*, also on the mechanism by which the excitation is transmitted through the plant tissue between the place of stimulation and that of response. If an element of the stem of *M. pudica* is stimulated, a state of excitation is propagated along the stem which on reaching the pulvinus causes the fall of the leaf system attached to it, and also the closure of the leaves; after some time the leaf system recovers from the effect of stimulation. Bose showed that an electrical disturbance of galvanometric negativity is recorded in a galvanometer, when its two terminals are connected to the pulvinus and to an indifferent point on the stem. On taking simultaneous records of both the mechanical and electrical responses on the same plate, both of them were found to be of similar character. According to Bose the stem leaflet system of a *M. pudica* plant respond to stimulation in ways very similar to those shown by a nerve muscle unit. Another plant which shows similar characteristics is *Biophytum sensitivum.*

In view of the great similarity in the responses to stimulation shown by both the plants and animal tissues, Bose concluded that the mechanism of transmission of stimulation in these plant tissues is similar to that in animal tissues, viz. by means of a propagated excitation current.

Investigations commenced by Ricca (1916) and followed up by other investigators appear to show that a chemical substance, extracted from the stem of mimosa plant, when introduced at the cut end of a Mimosa stem, produced similar closure of the leaf system as a physical stimulation, and further when a shoot of *M. spegazzinni* is cut across and the two pieces are connected by means of a short glass tube filled with water, then an intense stimulation of the lower side by means of flame is often followed by a fall of the leaf on the upper side. The conclusion was therefore drawn that a chemical substance released at the place of stimulation, and carried by means of the transpiration current, is responsible for the response shown by the pulvinus leaf system. Thus Ricca proposes a mass transport theory of the propagation of excitation in Mimosa, as opposed to the physiological theory of propagation by excitation current proposed by Bose.

The aim of the present paper is (i) to give a comparative study of the excitation phenomena in certain plant and animal tissues, to discuss how far they are dependent on physical and chemical factors, and from such study to arrive at some reasonable conclusions on the rôle played by a chemical mediator, the 'irritability substance' in the production of mechanical and electrical responses in Mimosa, (ii) to give an account of

the investigations undertaken to isolate the irritability substance, to determine its chemical composition and structure. This section includes an account of our own investigations on the subject. It will be seen that the conclusions reached by different investigators on the chemical nature of the substance do not agree with one another. This is due to the fact that more than one plant product can produce the same irritability effect and it is not easy to determine which of these is actually responsible for the propagation of excitation in the intact Mimosa plant. (iii) In the third part an account is given of our investigations on the chemical nature of the active substance whose presence in motile plant organs was established by Bose by means of staining and by microchemical tests. To anticipate the results of our investigations, we found the active substance to be similar to Crocin, a digentibiose of Crocetin found by Kuhn and Moewus (1938) in the motile flagella of the alga *Chlamydomonas eugamatos.*

It was further found by us that a solution of Crocin did not induce any mechanical response when introduced at the cut end of a Mimosa leaf system. Consequently the so-called 'active substance' is not identical with the 'irritability substance'. Chronologically this observation led us to follow up the investigations of Ricca and others.

(iv) Finally, the physiological actions both of the irritability substance and of the active substance, on motile pulvini are discussed.

PART I

PHYSICAL AND CHEMICAL FACTORS INVOLVED IN THE TRANSMISSION OF EXCITATION IN PLANT AND ANIMAL TISSUES

§2. In this section we shall first give an account of the general similarity of responses to stimulation observed by Bose in certain plant and animal tissues. Next we shall consider how far the phenomena of excitation in animal tissues can be explained on a purely physical theory; there is evidence to show that even in conduction of excitation in nerve muscle units it is necessary to postulate the presence of chemical mediators. Lastly, we shall consider how far the physico-chemical theory of transmission of excitation in animal tissues can provide us with a model to bridge over the antagonistic views on the transmission of excitation in Mimosa, viz. the purely physical theory proposed by Bose and the chemical theory of Ricca and others.

(i) *Similarity of response in certain plant and animal tissues*

The response to different modes of stimulation has been extensively studied in animal tissues. The forms of stimulation usually employed are, make or break of steady current or induction shocks of graduated intensities. The tissues generally experimented with are nerves, nerve muscle units and muscles. The muscles used are skeletal and smooth. The response in nerves is electrical, and is at present measured by valve tube amplifying arrangement and a cathode ray oscillograph. The response in a nerve muscle or a simple muscle unit is in addition mechanical. In plants like *M. pudica*, *B. sensitivum*, the mode of stimulation is either by graduated induction or electrothermal shocks. The response is either electrical or both electrical and mechanical. In *M. pudica* there is both a fall of the leaf system and closure of the leaves, while in *B. sensitivum* there is only a closure of leaves.

Summation effect.—If a nerve fibre is stimulated by a shock which is below the threshold intensity, the local excitability of the fibre remains above normal for a period which outlasts considerably the shock. Two or more such stimuli which are singly

ineffective may, if they are applied at sufficiently short intervals, cause stimulation. The summation time, i.e. the critical time interval above which no summation effect takes place is closely related to the other characteristic of the nerve tissue, the chronaxie. In a nerve smooth muscle unit, the amplitude of mechanical response is proportional to the intensity of induction shock times the frequency of stimuli, i.e. on the integrated intensity of stimulation. Amongst motile plants, *Mimosa pudica* petiole pulvinus unit behaves in a similar way.

All or none behaviour.—When a nerve fibre is stimulated electrically, excitation is produced only when the stimulus is above a minimum value. For stimuli above the threshold value, the potential wave transmitted is of a fixed magnitude and duration, which depends upon only the state of the fibre and not the strength of the stimulus which sets it into motion. If to the single nerve fibre a skeletal muscle is attached; the mechanical contraction of the latter is of fixed amplitude and is independent of the strength of the stimulus. Similar 'all or none' behaviour under stimulation is shown by the plant *Biophytum sensitivum* (Bose, 1913), e.g. when the intensity of stimuli is increased in the ratio of 0·1 to 1·0, the amplitude of mechanical response remains constant.

Stimulation by constant current.—A constant current above a threshold intensity flowing through a tissue, generally causes the tissue to respond only once at the cathode on making the current, and at the anode at break of current, i.e. when the current is made it initiates excitation near the cathode and inhibition in the anodic region; but the polar relations are reversed on breaking of the current. This effect is shown in nerves, nerve skeletal muscles, *M. pudica* and *B. sensitivum.*

Repetitive discharge (multiple response).—Sometimes under conditions which have never been clearly defined, closure of current may induce a repetitive response, the so-called closing or Pfluger's tetanus; even the breaking of a continuous current may be followed by similar repetitive response, the opening or Ritter's tetanus: shown by nerve, nerve skeletal muscle unit, *B. sensitivum.* Such multiple responses can also be initiated by the action of certain chemicals, injury effect in nerves; in *B. sensitivum* and in *M. pudica* under strong stimulation, and in the former by the action of light.

Electrotonus.—When a constant current passes through a nerve or muscle, it either inhibits or accentuates the transmission of excitation through it, depending on the direction of flow of the constant current. The region near the anode acts as a block to the current of excitation (anelectrotonus) while that near the cathode helps the passage of such currents (catelectrotonus). Expressed otherwise, excitation transmitted against the direction of the constant current is enhanced, and in the direction of the current the intensity is reduced; shown in nerve, nerve muscle units and in *M. pudica*, and *B. sensitivum.*

The above summary of the behaviour of plant and animal tissues under stimulation, indicates that the reaction in *M. pudica* is more akin to that in nerve and smooth muscle unit and of *B. sensitivum* to nerve skeletal muscle.

Physiological block.—Both in plants like *M. pudica* and *B. sensitivum* and in animal tissues, the conduction of excitation can be blocked and finally abolished by poisons, by anaesthetics like chloroform, ether, etc., and by the application of cold.

Theories of EXCITATION *in animal tissues*

§3. (*a*) *Physical theory.*—Since the transmission of excitation in a nerve muscle unit is always accompanied by an electric action current, many physiologists have tried to interpret the mechanism of transmission on purely physical concepts. Hill (1936), Rashevsky (1938) and others have proposed what is known as the two factor theory of excitation.

Hill calls these two factors excitation and inhibition factors of the tissue, and he does not make any further specification of their physical nature. Many observed effects including the excitations at cathode make and anode break can be interpreted on this form of the theory. The transmission of excitation from nerve to muscle can be explained on the analogy of the transmission of electric excitation between two circuits in resonance. This transmission is found to depend on (i) a tuning between the duration of nervous action potential and the time factor of muscle excitability, (ii) a certain ratio between the nerve action potential and the intensity factor of muscle excitability (rheobase). When a drug produces a physiological block it can be due to one or more of the following causes (i) changes in muscle or nerve chronaxie, (ii) depression of nerve action potential or increase in the muscular rheobase.

Limitations of the theory.—There are, however, certain phenomena observed during neuro-muscular transmission which cannot be accounted for on a purely physical theory (Monnier, 1936). Some of these are: (i) certain nerves produce on the attached effector muscles prolonged inhibitory effect. According to the physical theory permanent inhibition effect can only be produced by continuous repetition of impulses at a fast rate; (ii) artificial change of state of polarization in a tissue by a steady current (electrotonus); (iii) the law of summation of the effects of repeated sub-threshold stimuli; and (iv) production of repetitive discharges.

(*b*) *Chemical mediators in neuro-muscular transmission of excitation.*—Some of the gaps in the purely physical theory are bridged by the discovery of Loewi (1921) of a chemical mediator which is released at certain parts of a nerve muscle unit during the passage of a nervous excitation. In smooth muscles where all the muscle units are not innervated, transmission of nervous impulses from autonomic nerve endings is through the agency of chemical mediators like Acetyl Choline (Ac.Ch.), Adrenaline, etc. Some of these act as excitators and others as inhibitors. They are neuromimetic in so far as they can by their action directly produce the same muscular activity as is produced by a nervous impulse. Dale and Feldberg (1934) have shown that a chemical mediator, Ac.Ch., is active in the transmission of excitation from a motor nerve to its attached muscle. An injection of a small quantity of a dilute solution of Ac.Ch. into the arteries of a muscle gives rise to contractions of an asynchronous type. For the production of a quick twitch in voluntary muscles, there must be some mechanism for the disappearance of Ac.Ch. during the refractory period. An enzyme Choline Esterase (Ch.Es.) is found to be responsible for the deactivation of Ac. Ch. There is some difference of opinion as to the exact place where Ac.Ch. is released during stimulation of a nerve muscle unit. According to Lapicque, the location of the liberation is the muscle end plate; while according to Dale it is released from the endings of nerve fibres rather than from the end plate. It is believed to be held in some inactive form in the tissues, and is released therefrom by nerve impulse.

Later investigations (Lloyd, 1944) have shown that Ac.Ch. is not only liberated from nerve endings but also along the length of some nerves. While Ac.Ch. is momentarily released or activated during the passage of a nerve impulse, a deactivator Ch.Es. is always present in these tissues. From the estimation of the amount and activity of this substance present in these tissues, a more detailed explanation is produced of the initiation of nerve impulse (Bishop, 1946). The Ac.Ch. is released during stimulation, which depolarizes the tissue membrane by reducing its permeability. The Ch.Es. then decomposes the Ac.Ch., permitting the recovery of the initial membrane potential. The concentration of the esterase is estimated to be sufficiently high to permit removal of the ester during the time

of current flow; the flow to this depolarized region, of local current from the adjacent regions, is inferred to activate the ester in the adjacent regions and thus account for the propagation of the action current. Another enzyme has been found in nervous tissues capable of anaerobic synthesis of Ac.Ch. in the presence of Adenosine triphosphate.

Even now the precise function of Ac.Ch. in the complex series of events associated with or constituting the nerve impulse is not definitely known and as Foulton and Nachmanson (Lloyd, 1944) remark, 'there are certainly other factors and reactions involved in the propagation of nerve impulse, but newer investigations indicate that Ac.Ch. is an essential link in the generation of electrical change recorded during their activity'. Recently Nachmanson *et al.* (1946) have measured in the electrical organs of a number of specimens of *Electrophorus electricus*, the concentration of Ch.Es. responsible for the destruction of Ac.Ch. as well as the voltages of action potential. When the value of the recorded voltage, which was found to vary between 0·5 and 22·0 volt, is plotted against the Ch.Es. activity, the line which correlates the two apparently passes through the origin. This, taken along with other evidences, indicate a high probability of a connection between Ac.Ch. and nerve action potential and nerve conductivity. Recent investigations further show that a relation exists between Ac.Ch. concentration and electrotonus. Muralt (1937) has found that the passage of a steady current through nerves result in increasing the concentration at catelectrotonus and lowering at anelectrotonus of the Ac.Ch. content of nerves, whose excitability is initially high. This observation has been confirmed by Babsky (1946) who finds that electric polarization of nerve fibres of frogs, cats and dogs produce an increase of Ac.Ch. in the region of cathode and decrease in the region of anode, as compared to its content in controlled (unpolarized) parts of the nerve. Emulsions prepared from the portion of the nerve subject to the action of the cathode produced a much stronger contraction when applied to the dorsal muscle of the leech than those prepared from the anode or control parts.

Summarizing the results reported above, it appears that the following chain of events occur during the passage of a nervous impulse. The latter is propagated as an electric action potential along the nerve, activating Ac.Ch. on each surface area traversed. This results in a depolarizaton of the surface element and a consequent reduction of its equilibrium surface charge. This loss of surface charge causes a flow of local surface current from adjacent areas; at the same time Ch.Es. present deactivates the Ac.Ch. and brings back the area to its normal state. The adjacent area from which the equalizing local current flows undergoes a similar change of polarization, loss of surface charge, etc.*

The nervous impulse is thus propagated as an electric action potential and associated with it is a series of local chemical changes, but there is no bodily transport of the chemical mediator. The latter when directly introduced in a muscle unit produce contractions and is therefore neuromimetic. The summation and electrotonus effects can be satisfactorily interpreted in term of the chemical mediator theory.

* Many of the physico-chemical properties of surface membranes of living tissues can be reproduced in artificial oil-water interfaces, made by interposing between two electrolytic solutions, like two solutions of KCl, one saturated and the other of variable concentration, a water insoluble layer made of a mixture of a fatty acid like oleic acid and a phenol substitution product like cresol or guiacol. In this way potential differences due to concentration differences between the two electrolytic solutions can be measured. Certain alkaloids like atropin, pilocarpin, etc., in concentrations of the order of 10^{-6} produce, when added to the variable concentration electrolyte, a sudden drop of voltage of the order of ·005 V. It is reported by Beutner (1944) that introduction of acetyl choline induces a similar effect, but it is much more potent than the alkaloids, e.g. it requires less than 1/100 of the minimal concentration required by the alkaloids to bring about a sizeable effect, i.e. with a concentration of the order of 10^{-8}.

§4. *Transmission of excitation in Mimosa.*—In the introduction an account was given of Ricca's (1916) discovery that in *M. spegazzinni* a chemical substance, released at the place of stimulation and transported along the transpiration current is held to be responsible for the propagation of excitation. Snow (1924-5) further extended Ricca's observation and found that in *M. pudica* a slow rate of conduction took place in the stem, probably through the xylem at the rate of 15 to 28 cm. per minute. In addition there was a high speed conduction through the phloem of the petiole. The substance responsible for stimulation was not a protein, but of a simple nature, capable of diffusing through a collodin thimble without loosing its property. Ball (1927) showed that in the stem there were two modes of conduction. The normal one was a low speed conduction through the xylem, as found by Ricca and Snow; in addition there was another channel of conduction, in which the stimulus travels through the petiole at the rate of 200 cm. per minute. The normal conduction through the xylem is probably by means of the transpiration current; the highest rate of ascent of sap in Mimosa, according to Snow (Bose, 1926) is 18 cm. per min. For the high speed conduction Ball (1927) supposes that the stimulating substance instead of passing through the xylem merely causes contraction of the neighbouring cells which are possibly situated in the pith. This again releases further quantity of the hormone, and in this way a relay mechanism of a highly efficient nature is set up by which the hormone can pass in either direction in the plant and is independent of the water current. It will be noticed that the proposed mechanism is a chemical wave transmission, derivable from the physico-chemical transmission theory discussed in the previous section, by omitting the influence of local currents in releasing or activating the irritability substance. Fitting (1930), Umrath (1927–30) have also successfully investigated the stimulation effect produced by irritability substances extracted from Mimosa and other plants. Fitting observed repetitive closure and opening of Mimosa leaves at intervals of 7 minutes up to a maximum of five repetitions, when the cut end of the leaf system is dipped in an extract containing the irritability substance. Umrath finds such repetitive mechanical closure of the leaflets at intervals of 15 to 20 minutes, accompanied by electric pulsations of galvanometric negativity.

The interesting fact which comes out when the velocity of propagation of excitation in different portions of the Mimosa plant is measured, is the wide range of its variation, depending partly on the nature of the plant organ and its age. In Table I, we have collected the data, and have divided them into three groups.

Bose's criticisms (1926) of the transpiration theory of conduction are: (i) his inability to verify Ricca's observations in which he is supported by Kotesku's results; (ii) the speed of conduction of impulse up to a maximum of 2,400 cm./min. represents a different order of magnitude compared to the maximum speed of transpiration current in Mimosa, of 18 cm./min., obtained by Snow; (iii) his direct determination of the rates of translocation of an irritability substance and also of the stimulation released by it. He used (*a*) a stimulant which is also a staining agent like Methylene blue, and (*b*) a colourless stimulating agent like silver nitrate, the extent of whose translocation by sap movement can be detected by using a dectector like HCl. The results showed that while the chemical stimulant remained practically localized at the point of application, the impulse generated was transmitted to a considerable distance; (iv) then there are also the large number of investigations (Bose, 1907, 1913) which showed the similarity of transmission of excitation in Mimosa to that observed in nerve muscle units and the latter were at that time believed to be due only to the propagation of an electric action potential.

TABLE I

Group	Velocity cm./min.	Observer	Remarks
	15 to 28 up to max. of 52	Ricca, Ball, Snow ..	Stem conduction through xylem by transpiration; maximum speed of ascent of sap in Mimosa is 18 cm. per minute.
Slow	24 cm.	Bose ..	Stem and subpetiole.
	30–60	Fitting	
	up to 180	Bose ..	Thick petiole.
Medium	up to 200	Snow .. Ball	High speed conduction through petiole. No actual transport of active substance.
Fast	600 1,620 up to 2,400	Linbaum Umrath .. Bose ..	Thin petiole.

From these considerations Bose reaches the conclusion that the *normal* mode of transmission of excitation in Mimosa is neither hydrodynamical nor carried along by sap, but is of the same nature as the transmission of excitation in nerves and muscles.

A reasonable interpretation of the mutually contradictory observations and attempts at their explanations as reported above can be undertaken on the following lines:—

(i) At the place of stimulation a chemical substance, we shall call it the 'irritability substance', is released or activated. The state of electric depolarization induced by it, causes a flow of current from areas adjacent to it, thereby creating a state of excitation in these areas. This state of excitation is propagated as an electric excitation potential. Here there is no actual transport of the irritability substance. The laws of propagation are the same as those found to hold in the physico-chemical theory of transmission in nerve muscle units. The difference being that in *Mimosa* and in *Biophytum*, the amplitude of the action potential, the velocity of propagation are of a lower order of magnitude, while the refractory period and the period of repetitive responses are of higher order of magnitude.

(ii) The irritability substance has properties analogous to those of Acetyl Choline, in producing change of the state of polarization of the surface on which it is released or activated during the passage of the action current. It has also neuromimetic properties of inducing, single or multiple, mechanical and associated electrical responses in isolated Mimosa leaf system, whose cut end is dipped into a dilute solution of the substance.

The recovery from the state of depolarization is to be attributed by analogy to a hypothetical deactivator, which has not been isolated from *Mimosa* or *Biophytum*; alternatively the irritability substance may be transported away from its place of action by process of diffusion.

(iii) In addition to transmission of the state of excitation as an action potential with a high velocity of propagation, the irritability substance can also be propagated in the stem through the xylem along with the translocation current, its velocity of propagation is then of the order of 20 cm./min.

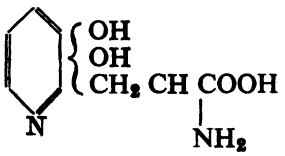
PART II

IRRITABILITY SUBSTANCE IN MIMOSA

§5. Ricca's discovery of the presence of an irritability substance in *M. spegazzinni* and Snow's discovery of a similar substance in *M. pudica*, have led to many attempts at isolation and chemical structure determination of this substance. The only criterion of the presence of this substance is that it must be water soluble and that when introduced to the cut end of stems of Mimosa leaf system which was initially kept with the stem dipped in tap water till the leaf has recovered from the initial shock of cutting, produces a collapse and subsequent recovery of the leaf system. Two methods for the isolation of the substance have been tried.

(1) Haberlandt (1890), observed under microscope, the formation of well defined mixed crystals when the drippings from cut ends of *M. pudica* were collected on a watch glass rubbed against it. From micro chemical investigations he concluded that it was some compound substance containing a glucoside.

Mölisch (1921) found it to be a substance of a phenolic nature; he however emphasized that this substance had nothing to do with the motility of *M. pudica* because the other motile plant *M. spegazzinni* does not yield a similar substance. Later on Jeny Renz (1936) proved conclusively that both Haberlandt and Molisch were working with impure samples. Chemical isolation and structure determination of the purified substance revealed that the latter was neither a glucoside nor a phenolic compound, but a 2–5 dihydroxy-pyridine to which an alanin residue is attached. The name of Mimosin was given to this compound. The substance is sparingly soluble in water and has an activity about 1/20 of that found by Fitting *et al.* with some amino acids. Nienburg and Tanbock (1937) confirmed Jeny Renz's finding and the following structural formula was proposed for it,



We followed up this investigation of Jeny Renz but we could not discover any sign of physiological activity on Mimosa cut stems using aqueous solution of Mimosin.

(2) Fitting (1930) and Umrath (1930) undertook to isolate the active substance by hot aqueous extraction from fresh leaves of Mimosa plant. Fitting in addition tried the effect of about 1,500 different chemical substances which are found in plants in extremely low dilutions. He found that only a few amino acids, Alanine, Glutamic acid, Glycocol, Amino-*n*-butyric acid, l-asparagin, when applied to cut ends of revived leaflets of *M. pudica* brought about the closure of the leaflets. But none of these amino acids were detected in the aqueous extract from Mimosa. Fitting concluded that the substance responsible for the closure of Mimosa leaflets must be 'a chemical substance of highly complex nature'. Umrath working with *Neptunia* isolated a very active substance, which resisted all attempts at crystallization. He identified it as an oxy-acid containing nitrogen.

§6. *Our own investigation.*—We took up the isolation of the irritability substance from warm aqueous extract of fresh *Mimosa pudica* leaves. At every stage of the purification the activity was checked by physiological tests. A series of preliminary investigations resulted in the following significant facts:—

(i) When fresh leaves of *M. pudica* is extracted with boiling water, it is of yellowish tint which on keeping darken to reddish pink colour. Colour of the solution

can be completely removed by boiling with activated charcoal. On evaporating down to syrupy consistency the colour reappears. The aqueous solution of the syrup gives positive Mimosa test.

(ii) If aqueous extract of *M. pudica* is treated with twice its vloume of 95% alcohol, white voluminous precipitate, mostly of protein and inorganic salts, are thrown down. The precipitate was sparingly soluble in water and its aqueous extract gave negative Mimosa test.

(iii) When freshly prepared leaf extract is kept at the room temperature bacterial and fungal contamination take place with resultant loss of activity. It can be kept for indefinite period by the addition of an antiseptic such as toluene or alcohol. It can also be preserved in cold.

(iv) Umrath's methods of isolation of the active substance involved successive precipitations of the active substance as salts of heavy metals, e.g. lead and mercury salts and subsequent removal of the metals as sulphide which resulted in lead contamination.

A. *Attempt at isolation of the irritability substance by extraction with neutral solvents*

In order to avoid any drastic chemical treatment, the removal of associated impurities of the solution was tried by extracting it with different inert solvents, in the following order:—

Toluene, Benzene, Petroleum ether, Chloroform, Ether, Amyl alcohol, Butyl alcohol. After solvent extraction, the aqueous extract was concentrated under reduced pressure. The activity of the solution was found to be unaffected.

12½ lb. of fresh leaves of *M. pudica* were extracted with 12 litres of distilled water by refluxing it for about an hour on a water bath. After filtration through a porcelain Buchner without a filter paper the yellow coloured liquor was concentrated down to 3 litres under reduced pressure, the temperature during concentration was kept between 35°–40° C. On keeping the concentrated liquor in a refrigerator overnight, a brown precipitate mostly of phlobaphene was thrown down. Filtered. The concentrated liquor again developed the caramel colour. Two volumes of rectified spirit were added, protein, pectinous material and inorganic salts were thereby removed. The 9 litres aqueous alcoholic solution was kept as stock solution from which 2 litres were again concentrated under reduced pressure to 200 c.c., which was successively extracted with toluene, benzene, petroleum ether, ether and chloroform. Duration of each extraction was 10–12 hr. It was then decolourized with activated charcoal and concentrated down to 50 c.c. The solution was separated in two portions, one was kept in a refrigerator and the other in a vacuum desiccator over sulphuric acid. Even after several weeks no tendency to crystallization was observed in either of the two portions. It was then taken up and extracted in liquid extractor with amyl and butyl alcohol. Both the substances removed impurities of oily nature. After removal of the solvents the aqueous solution was divided in two portions. One as such was kept in refrigerator and to the other drop by drop alcohol was added till turbidity appeared and then it was kept in cold in a stoppered conical flask. After 3 weeks fine granular precipitate was found to settle at the bottom of both flasks. The supernatant liquid was poured off. The crystals were dissolved in a minimum quantity of water and recrystallized. The aqueous solution of recrystallized substance was inactive whilst the mother liquor was active.

The mother liquor gave 'Ninhydrin test', indicating the probable presence of amino acid group. A bluish green precipitate was observed with ferric chloride, indicating the

presence of a phenolic hydroxyl group. After being hydrolyzed with dilute mineral acid, it was reduced with Fehling's solution, showing the presence of a glucoside. The solution was acidic to litmus and gave effervescence with sodium bicarbonate, indicating the presence of a free carboxalic group. As the degree of purity of the solution could not be vouchsafed, it is very difficult to predict the exact chemical nature of the substance in it.

B. *Attempt of isolation of irritability substance by fractional precipitation*

To an aqueous solution (100 c.c.) from which alcohol had previously been removed by vacuum distillation, lead acetate solution was added drop by drop till the precipitation was complete. Thereby tannin, plant mucilage and proteinous matters were completely removed. Filtered under suction. To the filtrate, basic lead acetate solution was added till the solution was alkaline to litmus. The basic lead acetate precipitate was suspended in water, warmed, and H_2S was passed, till whole of the lead was removed as lead sulphide. The solution was separated from lead sulphide by filtration. H_2S was removed by aspirating CO_2 through the solution. The bright yellow coloured solution was concentrated down 8 to 10 c.c. under reduced pressure. It was then kept in a vacuum desiccator for a couple of days. A syrupy mass of red colour was obtained. On addition of a minimum quantity of water dirty flocculent precipitate was obtained which was removed by filtration. The solution was extremely active.

It gave a bluish green colouration with ferric chloride. It was negative to Ninhydrin reaction. It reduced Fehling's solution before and after hydrolysis. It was acidic to litmus and gave effervescence with sodium bicarbonate solution.

We concluded obviously that certain amount of glucose and tannin remained associated with the active substance. So the fractional precipitation with lead acetate and basic lead acetate was repeated thrice but it did not improve the matter. The test of tannin persisted. Moreover, starting even with 12 lb. of fresh leaves of *M. pudica* the yield of the final product was extremely small. There was no tendency to crystallization of the syrup even when it was kept in a vacuum desiccator or in a refrigerator for 6 to 10 weeks.

C. *Isolation of Tannin from dried leaf powder of M. pudica which was found to be an irritability substance*

100 gm. of dried leaf powder was packed evenly in a glass percolator tube. About 1 litre of rectified spirit was allowed to percolate through the powder very slowly. After about 6 hr. the rectified spirit which ran down became colourless. The deep green coloured alcoholic extract was concentrated down to a syrupy paste under reduced pressure. Last trace of alcohol was removed under vacuum. About 200 c.c. of distilled water was added to the greenish black mass which adhered to the bottom of the flask. Filtered through a filter paper. Chlorophyll and resinous mass remained adhering to the filter paper and the filtrate was a red coloured clear liquid. The filtrate on keeping overnight in refrigerator deposited brownish red precipitate at the bottom of the flask, which on testing was found to be phlobaphene. Filtered. The filtrate was extracted with ethyl acetate for about 6 hr. in a liquid extractor. The ethyl acetate extract was then evaporated to complete dryness under reduced pressure. The last trace of ethyl acetate, which adhered to the spongy brown mass, was removed by the addition of few drops of water and distilling off under reduced pressure. A very brittle spongy mass was thereby obtained. It was powdered by means of glass rod and kept in a vacuum desiccator over sulphuric acid. A small quantity of the brown coloured powder was dissolved in minimum quantity

of water. Filtered. Slight insoluble red precipitate soluble in alcohol was left behind. It gave all the tannin test:—

(i) It precipitated gelatine; (ii) gave greenish black precipitate with ferric chloride; (iii) with concentrated sulphuric acid gave red colouration at the junction; on shaking dirty greenish precipitate was obtained; (iv) formaldehyde HCl on addition gave a pale-yellow precipitate which when refluxed over free flame for half an hour turned pink.

All the test given above indicate the presence of catechin tannin. Further investigations are being undertaken in order to ascertain definitely whether it is mixture of tannins or a single tannin, or a substance closely associated with tannin.

§7. Three different methods have been used to isolate the irritability substance from the Mimosa plant which lead to the following results:—

A—Extraction by means of neutral solvent result in a solution which give the Mimosa test and also contain (i) an amino acid group, (ii) a phenolic hydroxide group, and (iii) a free carboxalic group.

B—Successive precipitation and purification of the substance by lead acetate method, indicate that no amino acid is associated with the irritability substance, but the latter contains a certain amount of tannin material, and a glucoside is associated with it, resulting in an end product of a syrupy substance not easily crystallizable.

C—Use of a direct method, for the isolation of the tannin group, indicates that the irritability substance probably belongs to the catechin group. It is not certain whether the latter is a single tannin or a mixture of tannins or a substance closely associated with tannin.

Note added in proof.—We have recently received a paper from G. Hesse 'Uber die Natur der Erregungssubstanz Von *M. pudica*' (*Biochem. Z.*, 303, 152, 1939) in which it is mentioned that K. Umrath *et al.* (*ibid.*, 284, 247, (1936) and *Protoplasma*, 31, 454, (1938)) have obtained from *M. pudica*, using Fitting's technique, an amorphous concentrate which under favourable conditions give the Mimosa test at a concentration $1 : 5.10^8$. The substance behaves as an oxy-acid or oxy-amino acid with a molecular weight between 300 and 450. This substance appears to have properties similar to that isolated by us using method A.

We have devised a test which is more sensitive than the one used by Fitting, for determining the limiting concentration at which a substance will give the Mimosa test. We find that with a crude extract from Mimosa, the limiting concentration is $1 : 10^9$, while with the tannin body isolated from Mimosa by method C, we have found the test for concentrations up to $1: 10^4$. This, however, does not represent the lower limit. The investigation is proceeding.

PART III

NATURE OF THE ACTIVE SUBSTANCE PRESENT IN THE PULVINI OF MOTILE PLANTS

§8. It was found by Bose (Motor Mechanism of Plants, 1928) that pulvini of plants with motor organs exhibit characteristic differences both as regard their sensitiveness as well as in the rate and extent of their contractile movements. Three types of plants with pulvinated joints, all belonging to the Leguminacae group, are distinguished by him—Active, Semi-active and Inactive.

Table II is a reproduction from page 59 of Bose's book.

TABLE II

Rate of Contractile Reaction of Active, Semi-active and Inactive pulvini on application of maximal stimulus

Specimen	Period of maximum fall (in seconds)	Angular movement of fall (in degrees)	Rate of fall per second (in degrees)
Mimosa ..	1·5	100·0	66·0
Neptunia ..	180·0	15·0	0·08
Erythrina ..	480·0	0·8	0·002

According to Bose, the intensity of motor response, i.e. the rate and extent of contraction of the pulvinus is dependent upon (i) the supply of oxygen to the plant, (ii) on the tonic condition of the plant, and (iii) on some specific property of the motor organ which varies with the nature of the plant. Even favourable tonic conditions cannot make the semi-active tissues contract as rapidly as an active one. The rapidity of contraction appears to be due to the presence of some other factor, viz. a special modification of the protoplasmic content of the active cells. The localization of the active contractile cells in a petiole pulvinus preparation of *M. pudica* was undertaken by Bose. For this purpose he used pairs of stains like Haematoxylin and Safranin, etc. It was found that while the content of the cortical cells remained unstained, those of the pulvinus were deeply stained; the latter were more numerous and compactly arranged in the lower half, while in the upper half they were relatively few and scattered in their distribution. Bose found that these pulvini could be arranged in a scale of stainability of their active cells, and that this scale followed closely the scale of excitability of the corresponding pulvini. He concluded that the stainable substance is directly related to the contractibility of the tissue, in fact it is the 'Active Substance'. Microchemical tests showed that while the latter is highly oxidizable, it is neither a fat nor a lipoid substance; it contains unsaturated carbon in double or triple bonds. Prof. Hans Mölisch, who was working in the Bose Institute at this time, used his well-known test for the detection of phloroglucine compounds on sections of pulvini of *M. pudica* and found them to be stained deep crimson. He concluded that the active substance detected by Bose was probably a phlorogluco tannoid compound. The reagent used by Molisch for this test is a solution of paradimethyl amino benzaldehyde $C_6H_6N(CH_3)_2.CH.O$ in sulphuric acid—we shall designate it as Molisch's test 2. By Mölisch's test 1 we denote the test used by him for the detection of carotinoid compounds in plant tissues.

§9. Our interest in Bose's observations was revived by coming across reports of recent important investigations of Kuhn and Moewus (1938) on the rôle of a certain class of crocetin compounds in the life processes of the alga *Chlamydomonas eugamatos* and of other allied strains. Crocetin, $C_{18}H_{28}(COOH)_2$ belongs to the carotinoid group of compounds, with conjugated double bonds. According to Moewus this alga develops flagella which become motile (i) when kept in water and exposed to visible light, or (ii) in the dark when placed in a glucose solution and supplied with O_2, or (iii) in the dark in the absence of O_2 when placed in a filtrate from already motile cells. On examination, the concentrated filtrate was found to contain a crocetin compound which is either Crocin (a digentibiose of Crocetin) or similar glucosides of Crocetin. Biological tests with Crocin obtained from saffron showed that this colouring matter was able, exactly like the natural

motility producing substance, to develop motile flagella in the absence of light. Another compound, Crocetin dimethyl ester $C_{18}H_{22}(COOCH_3)_2$ in certain combinations of its cis- and trans- forms, is capable of developing male and female gametes. In view of Bose's conclusion regarding the presence of unsaturated carbon bonds in the active substance found in motile pulvini of *M. pudica* and other plants, it appeared worth while to investigate whether the latter belonged to the carotinoid group, and in particular to the crocetin group.

The investigations reported here were planned to be carried out as follows: (i) to repeat and confirm Bose's original observations, using his staining technique; (ii) to find out whether carotinoid compounds were present in the motile plant pulvini, using Molisch's test 1; (iii) assuming the carotinoid compound to belong to the crocetin group, to isolate it by the method of Stoll and Willstätter (1913), using neutral solvents. At the same time different crocetin compounds, found in Indian saffron, were isolated and crystallized. These were intended to provide pure samples with which similar compounds isolated from Mimosa and other plants could be identified by comparison; (iv) Molisch's investigation using his solution 2 was repeated in order to verify the latter's conclusion that the so-called 'Active Substance' found by Bose were compounds of Phloroglucin $C_6H_3(OH)_3$, which also contain conjugated double bonds. An unexpected result was obtained, viz. the test solution 2 not only produced the anticipated deep crimson coloration in the pulvini sections, but also revealed the presence in the latter of glucosides of Crocetin.

(i) and (ii), as a preliminary Bose's original investigation using differential stains, were repeated and confirmed by Mr. A. K. Adhikari. The latter then carried out Molisch's test 1 for determining the presence of carotinoid compounds in the active pulvini of the different plants investigated by Bose. Surprisingly large quantities of carotene crystals were found in the primary and secondary pulvini of *M. pudica* and in other motor plant pulvini. It was also noticed that the density of distribution of carotene crystals, observed in active cells of the different plant pulvini treated as above, followed roughly the same order of distribution as obtained by Bose with his staining method. The drastic chemical method used in this test reduces the different carotinoid compound to the same end product, and therefore the method is not suited to reveal the chemical composition of these compounds as originally present in the plant cells.

Chemical methods of isolation of Carotinoid compounds from Indian saffron and pulvinus of M. pudica

§10. For comparative physiological studies, representative samples of pure Crocin, cis- and trans-Crocetin dimethyl ester were essential. These compounds had been isolated by Karrer and Solomon (1927), Kuhn and Winterstein (1933, 1934) from saffron, obtained from Southern France, Spain and Asia Minor. We have isolated the pigments from Kashmir saffron. Our methods of isolation of Crocin, Crocetin, cis- and trans-Crocetin dimethyl ester were mostly based on the findings of Karrer and Kuhn (*loc. cit.*).

Crocin.

40 gm. of saffron was dried for 7 hrs. at 80–90°C. in an air oven. It was then powdered; loss in weight was about 4 gm. 36 gm. of saffron was first extracted with petroleum ether (b.p. 60–80°C.) for 18 hrs. in order to remove fat and lipoid bodies. It was then extracted with absolute ether in a Soxhlet apparatus with a calcium chloride guard-tube for 10 hrs. whereby picro-crocin and some resinous substances were removed. After petroleum ether and ether extraction, the adhering solvent was removed from the

saffron residue by spreading it in a thin layer on a porcelain basin. The residue thus obtained was extracted with 80 c.c. of MeOH (80% by vol.). The extraction was conveniently carried out by refluxing it on a water-bath for about half an hour; filtered under reduced pressure. The filtrate, a deep orange-coloured solution, was treated with so much methanol as to make the concentration of methyl alcohol to 90%. It was then kept in a refrigerator. After six weeks, a violet deposit was found to settle at the bottom of the flask. It was impure Crocin associated with some insoluble and resinous matter. For purification it was treated with the least amount of pyridin, by which a large quantity of associated impurities remained undissolved, filtered. The pyridin solution was poured into absolute ether. The red resinous precipitate was treated several times with absolute ether in order to remove last traces of pyridin. It was then kept after dissolving in a minute quantity of methanol in a calcium chloride desiccator. After about a week, deep orange-coloured crystals were obtained, melting at 212°C. Karrer assigned the melting point of pure Crocin as 185°C., but Kuhn found the melting point of twice recrystallized Crocin as 215°C.

Cis- and trans-Crocetin dimethyl ester.

The residue left after extraction with 80% methanol was extracted twice with 70% methanol. And the 90% methanol extract of Crocin preparation was mixed with second and third extracts. To the whole of the extract so much N-NaOH solution was added that every litre of it contained 50 c.c. of normal alkali. After allowing it to stand at room temperature for about 3 hrs. an orange-coloured precipitate was found to settle at the bottom of the flask. Filtered. To the filtrate so much water was added that the concentration of methyl alcohol was lowered to 50%. A second crop of precipitate was obtained. Filtered. Both the precipitates were combined together and shaken with 30 c.c. of a mixture of MeOH : Ether (1 : 1) in a mechanical shaker for about an hour. Filtered. The residue was then shaken twice with 1 lb. of ether in the same manner; the whole of Crocetin dimethyl ester went into solution in ether. The ethereal solutions were combined together and was concentrated to $\frac{1}{3}$ its volume. Trans-Crocetin dimethyl ester crystallized out. It was recrystallized from pyridin.

The ether was completely evaporated. The residue was boiled with small quantities of methanol. Filtered. The filtrate on cooling deposited almost pure cis-Crocetin dimethyl ester. The methanol mother liquor was used for further treatment of the residue. By which another crop of crystals of cis-Crocetin dimethyl ester was obtained. The whole crystals were then twice recrystallized from petroleum ether.

Trans-Crocetin dimethyl ester crystallized from pyridin in beautiful, orange-coloured, diamond-shaped crystals melting at 222°C.

Cis-Crocetin dimethyl ester crystallized from petroleum ether (60–80°C.) in long yellowish prism melting at 141°C.

Crocetin.

The residue left after treatment with ether was extracted with chloroform. Filtered. The sodium salts of trans-Crocetin and Crocetin monomethyl ester were left behind. The mixture was boiled with acetic acid. Sodium salt was decomposed. The mixture of Crocetin and its monomethyl ester was boiled with 10% alcoholic potash for an hour, by which Crocetin ester was hydrolyzed. The potassium salt of Crocetin and Crocetin monomethyl ester remained behind. It was allowed to cool, and filtered under suction. Boiled with 20 cm. of acetic acid cooled and filtered. The raw Crocetin was crystallized out of pyridin, m.p. 285°C.

In the literature of saffron pigments, mention of yield is very scarce; only source of the saffron, season and region from which it is collected are mentioned. Kuhn (1933) had given the yield of different pigments. Indian saffron is richer in these pigments, and we have actually found the yield of cis-Crocetin dimethyl ester as 0·2%, whilst Kuhn reports a yield of 0·1% in European saffron.

Isolation of Crocetin from the primary pulvinus of M. pudica

§11. Only primary pulvinus of *M. pudica* was collected. It was dried at 40°C. for three days and powdered. The powder weighed 15 gm. It was extracted with 100 cm.^{3} (80% by vol.) of acetone. All the chloroplast pigment went into solution. From acetone it was then taken up in 60–70 cm.^{3} of petroleum ether (b.p. 60–80°C.) by the addition of much water to the acetone petroleum ether mixture. It was then washed twice with water. The petroleum ether was then shaken twice with acetone (80% by vol.). The acetone was removed by washing it four times with water. The petroleum ether solution contained all the pigments. By shaking it thrice with 60 c.c. of methyl alcohol (80% by vol.) the zanthophyll was removed. In petroleum ether we had carotin and chlorophyll. Last traces of acetone and MeOH were removed by washing thrice with 60 c.c. water by which petroleum ether solution became turbid. Chlorophyll separated out. The petroleum ether solution was dried over anhydrous sodium sulphate. The solution is filtered, shaken with about 20 gm. of talcum powder. Filtered through a layer of talcum powder to remove chlorophyll: complete removal of chlorophyll was not achieved. So chromatographic absorption over powdered sucrose was resorted to. After allowing the petroleum ether solution to pass through a column (10 × 1 cm.) of powdered sugar, we obtained a solution which contained just traces of chlorophyll, which was removed during crystallization. The volume of petroleum ether solution was then reduced under vacuum. The only residue (1 to 2 c.c.) was poured in about 15 cm.^{3} of rectified spirit. A turbid semi-solid mass settled at the bottom of the flask. It was kept in a refrigerator. Next day it was taken out and triturated with petroleum ether and the supernatant liquid was poured off. It was then washed with a mixture of 2 vol. of petroleum ether and 1 vol. of alcohol and kept for about a month in a refrigerator. The quantity left over was not sufficient for further purification. In order to find whether any crystals were present a little quantity of it was put under the microscope. Crystals were seen which resembled exactly with those micro-photographs of Crocetin crystals given in Klein (1932).

Whole leaf (20 gm. of dry powder) of *M. pudica* was extracted in the same way as in the case of primary pulvinus. Here also a few crystals of Crocetin was observed though in association with other impurities.

Total carotinoid compound in green leaves according to Willstätter and Stoll is of the order of 0·1 to 0·2%.

The above investigation shows that Crocetin can be isolated from both the pulvinus and leaves of *M. pudica* by neutral solvent extraction. It is not indicated whether besides Crocetin, other crocetin compounds are also present in the plant tissues. Investigations carried out by means of *in situ* staining of cut sections of pulvini give further information on this point.

In situ test on sections of plant pulvini, using Molisch's solution 2

§12. Having established that Crocetin compounds occur in the pulvini and leaves of *M. pudica*, the question arose whether the deep crimson coloration produced by application

of solution 2 to cut sections of pulvini of *M. pudica*, as observed by Molisch, was due to an interaction between the Crocetin compound and paradimethyl amino benzaldehyde. For this purpose, Molisch's investigations were repeated. On carrying out the test on a glass slide and observing the reaction under a microscope, the expected deep crimson coloration was observed in sections of pulvini of many leguminous plants, some of which are not motile. In addition, a new effect was observed, not previously reported by Molisch, viz. from the treated section of the pulvini an orange red coloured liquid flowed out, from which crystals separated out identical in shape and colour to crystals of trans-Crocetin dimethyl ester which were previously isolated from Indian saffron and referred to in a previous paragraph. In Table III we give the results of our observations on sections of different plant pulvini.

TABLE III

Class	Specimen	Reaction under Molisch's solution 2		No. of observations
		Crimson stain of section	Formation of coloured crystals	
Active ..	Primary pulvinus of *M. pudica.*	+	+	17
	Secondary pulvinus of *M. pudica.*	+	+	15
	Secondary pulvinus of *M. spegazzinni.*	+	+	5
Semi-active ..	Secondary pulvinus of *Neptunia.*	+	+	5
	Secondary pulvinus of *Averrhoa.*	+	+	3
Feebly active ..	Primary pulvinus of *M. spegazzinni.*	+	−	5
Inactive ..	Primary pulvinus of *Averrhoa.*	+	−	9
	Primary pulvinus of Erythrina.	−	−	3
	Primary pulvinus of Phaseolus.	−	−	3
Pulvinule of *Desmodium gyrans* leaflet (pulsate spontaneously).		+	+	2

Thus we find that all motile plant pulvini show, under Molisch's test 2, not only the expected deep crimson staining, but also the formation of orange red coloured crystals. To confirm our tentative conclusions, we added to an aqueous solution of Crocin, obtained from Indian saffron, a few drops of Molisch's solution 2. No deep red coloration was obtained, but when observed under a microscope, a few characteristic deep orange-coloured, rhombic crystals were observed. On the other hand, the same solution, when added to glucose, sucrose and other polysaccharides, did not result in the production of these characteristic crystals. It appears that Crocin or similar other glucoside compounds of Crocetin are present in the motile plant pulvini, and the following chemical reaction is responsible for the appearance of the orange red crystals, viz. the two glucoside groups, similar to digentibiose attached to Crocetin nucleus in Crocin-like molecules, suffer acid hydrolysis when treated with solution 2, and are replaced by the two methyl groups from *p*-dimethyl amino benzaldehyde molecule to form trans-Crocetin dimethyl ester which, being insoluble

in water, crystallize out from the reacting solutions. In Fig. 1 microphotographs of crystals, all orange red in colour and rhombic in shape, are shown representing crystals of

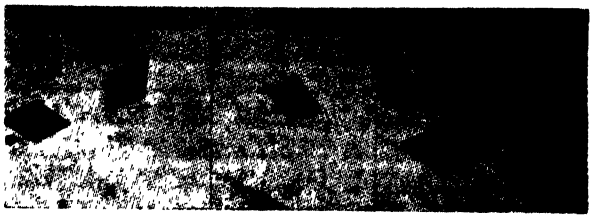


FIG. 1. Microphotographs of trans-Crocetin dimethyl ester crystals (magnification 56): (A) isolated from saffron, (B) formed *in vitro* by action of solution 2 on Crocin isolated from saffron, and (C) formed *in situ* by addition of solution 2 to a section of pulvinus of *M. pudica*. The faint appearance of the crystals in (C) is due to faulty exposure.

trans-Crocetin dimethyl ester (A) isolated from Indian saffron, (B) formed *in vitro* by the action of solution 2 on Crocin isolated from the same source, and (C) formed *in situ* by the addition of solution 2 to a section of pulvinus of *M. pudica*.

From the above observation the following inferences can reasonably be drawn: (i) that Molisch's solution 2 is a suitable reagent for *in situ* detection in sections of plant organs, of Crocin or similar glucosides of Crocetin, and (ii) such glucosides are present in the active pulvini of motile plants and they are probably identical with the unsaturated carbon compound detected in them by Bose and designated by him as 'Active Substance'. The method described does not at present permit a quantitative determination of the amount of the substance present in the pulvini.

It is not clear what the rôle of the Crocin-like active substance in the motor activity of the pulvinus is. The presence of glucose molecules in the Crocin-like substance suggests that the latter is responsible for the supply of energy required for mechanical work; but the rôle of the Crocetin rest molecule in the process is still obscure. Kuhn and Moewus (Sonneborn, 1942), from a study of the occurrence of cis- and trans-dimethyl ester molecules in the male and female gametes in the different species of *Chlamydomonas*, which roughly follow the ratio of Bergmann-Niemann numbers for the occurrence of amino acids in protein molecules, viz. $2^m \times 3^n$, when m and n can have values 0, 1, 2, 3, etc., have suggested the hypothesis that the Crocetin compounds act as prosthetic groups which are attached to the amino acids of certain enzymes. From this it may be inferred that the glucose molecules in the Crocin-like substrates become attached to the corresponding enzyme through the Crocetin rest molecule. It is known (Flint, 1942) that maximum phototropic effect occurs in plants, with or without chlorophyll, in the region of maximum light absorption by carotinoid compounds.

PART IV

PHYSIOLOGICAL EXPERIMENTS UNDERTAKEN TO FIND THE RÔLE OF THE CHEMICAL MEDIATOR IN THE PROCESS OF TRANSMISSION OF STIMULATION IN M. PUDICA

§13. (*a*) Cut-ends of two branches of two different plants of *M. pudica* were joined together by means of a capillary glass tube filled with water. After the plants had returned to their normal states, one of them was electrothermally excited and in 10% of cases the

other plant responded by drooping of leaves. The time interval between stimulation and response varied from 40 minutes to 2 hours.

After having obtained a positive result, the water contained in the capillary tube was collected on a glass slide and solution of *p*-dimethyl amino benzaldehyde was added to it to see if the water contained some substance which gives red coloration. But no such coloration could be detected under microscopic examination.

(*b*) Haberlandt (1896), Jeny Renz (1936) and others have collected the liquid oozing out of cut-stems of *M. pudica* from which a fairly soluble and easily crystallizable substance can be isolated. Jeny Renz found that this substance gave the Mimosa test. This line of investigation was repeated. Sap was collected from the cut-ends of stems and pulvini of *M. pudica* and applied directly to the cut-ends of shoots and pulvini mounted on small clamps. More than 40 experiments were conducted, but not a single one gave the Mimosa test. In order to be sure, more experiments were conducted in a different way. Instead of taking cut-stems or isolated pulvinus, the delicate portion of the stem towards the tip of an intact healthy plant was split up longitudinally with a sharp pointed knife. The incision was less than a quarter of an inch in length. A slender wedge was inserted through it, so that a slight opening is made between the two halves of the split up stem like the eye of a needle. Sap oozes out of the cut-end and soon dries up to form a thin layer over the wound. To prevent this layer formation, a drop of water is added to the opening, which remains suspended on it until the plant recovers from the shock. This suspended drop of water also helps in hastening the recovery of the plant. By this method the effect of an irritability substance, however feeble, can be detected easily and with greater consistency. Due to the variability in the condition of the plant, depending on light, temperature, moisture content of the atmosphere, etc., it is difficult to obtain precise quantitative results by one and the same physiological method in different experiments. There is, however, a greater chance of variation in results obtained with experiment on isolated parts of the plant. With the splitting method, a drop of sap taken direct from the cut-end or dried up sap taken in solution of distilled water was applied to the wedged opening of the stem. In 23 experiments not a single pulvinus responded to this treatment. Thus we have not been able to confirm the observations of Jeny Renz. Methods of injecting solution were also tried but without any success.

(*c*) Two potted plants of *M. pudica* were placed side by side. One of the branches from each plant was cut transversely at the tip of the shoot. As soon as the sap oozes out of the cuts, both the ends were joined together by pressing them slightly against each other, and in order to keep the cut-ends moist, a drop of water was kept suspended at the joint. After the plants had recovered from shock, one of the branches was given an electrothermal stimulation. Transmission of stimulus from one plant to the other was not observed in any one of the experiments.

(*d*) No transmission of stimulus was observed even when two cut-ends remained a few mm. apart and joined by a suspended drop of water.

(*e*) Two branches, belonging to two separate potted plants, were slantingly cut and fitted together as one stem and tied around with thread. After recovery, thermal shock was applied to one of the branches, but the stimulation did not travel from one end to the other.

(*f*) Two isolated pulvini of *M. pudica*, with leaflets, were mounted on two arms of a minute capillary U-tube filled with water. After recovery, shock was given to one of the leaflets which collapsed immediately, but the other remained open as in normal plant. More than 20 experiments were carried out without any positive results.

(*g*) In order to find out whether the crystals of Crocin, whose presence in motile pulvini is revealed by Molisch's test 2, have any physiological activity, experiments were carried out using different methods. Cut-stems of short length with leaves intact were mounted on small clamps attached to short stands. The cut-ends were dipped in water in small glass vessels. After recovery, the clamp with the plant was slightly raised on the stand without the least disturbance to the plant and the water vessel removed. Then a minute test-tube, half-filled with dilute aqueous solution of Crocin isolated from Indian saffron, was placed in proper position and the clamp was lowered slowly till the cut-end dipped in the solution in the test-tube. Even when the cut-end was kept dipped in the solution for a considerable period of time, no irritability effect was observed. Cut-ends of isolated pulvini with leaflets intact were treated in a similar manner but without any effect.

(*h*) Some microcrystals were obtained from the extracts of leaves and stems of *M. pudica* following the chemical method employed by Stoll and Willstätter, which enables the separation of Carotinoid compounds from chlorophyll and zanthophyll. These crystals were found to be identical with the photographs of Crocetin crystals. To determine if these were the irritability substance, the crystals were dissolved in distilled water and applied to the cut-ends of pulvini and in the opening of the split up stem, but the motile organs of the plant gave no response at all.

(*i*) Green leaves and stems of *M. pudica* were cut into pieces. They were smashed in a mortar with distilled water. The juice was squeezed out and properly filtered. With a small pipette this cold water extract was then applied to the cut-ends of pulvini and the split up stems of *M. pudica* just after recovery. Most of them responded to this treatment within a short time varying from 2 to 8 seconds. Some of them gave multiple responses. About 90% of the experiments gave positive results. More than 300 experiments were carried out.

(*j*) Cold water extraction of some other plants, such as young shoots of Mango, Jamrul, Jam and the semi-active plants such as *Neptunia*, *M. spegazzinni*, were also applied to the split up eyelets of *M. pudica*. They showed the Mimosa effect in almost all the cases and sometimes more vigorously than with an extract of *M. pudica*.

(*k*) The final solution obtained by chemical method, which gave all the tests of tannin, was tried on *M. pudica*. A new method of experimentation was adopted in this case. Instead of taking cut-stems or isolated pulvinus, short longitudinal incisions were given with fine pointed knife in the petioles just beyond the pulvini in intact plants. After complete recovery within 5 to 8 minutes, a drop of the solution was applied to the incision. Within 2 to 6 seconds the pulvinus drooped down and the leaflets collapsed. In about 90% of the cases positive results were obtained.

(*l*) In order to investigate if the excitation current travels through a preferential path, i.e. a particular layer of cells, some experiments were undertaken. Stems of intact plant of *M. pudica* were stripped off the cortical layer about one inch in length. After about 15 to 20 minutes when the plant came back to its normal condition, thermal shock was applied to the exposed woody stem of the stripped off portion. Excitation travelled first upwards and then throughout the plant within 5 to 10 minutes. All the experiments conducted gave positive results.

(*m*) Stems of *M. pudica*, completely stripped off of barks, were collected in a mortar and smashed with distilled water. The filtered extract was applied to the cuts of leaves and stems of a potted plant. In 7 cases out of 13 experiments the pulvini responded within a second or two and in 3 cases leaflets collapsed within 5 to 8 seconds. One stem and two pulvini gave no response.

(*n*) It has been observed that in a single leaf of *M. pudica*, the pulvinus or different leaflets could be excited individually with a slight touch at the motile zone. Here the question of transmission does not come in. Still to get a sure test of it only the primary pulvinus without the petiole, $\frac{1}{8}$ of an inch in length, was mounted on a pin-point. A slender glass string was attached to it in the position of the petiole to act as an index. After having kept it in moist condition the bent pulvinus muscle came to a horizontal position, i.e. it recovered from the shock of the cut. Then it was electrothermally excited; the muscle bent down again showing the displacement of the glass index. By careful manipulations four experiments were successfully conducted. This showed that the irritability substance is stored in the pulvinus in an inactive form.

SUMMARY.

The present paper deals with a series of investigations designed to elucidate the nature of the processes intervening between the stimulation of a part of a sensitive plant like *Mimosa pudica* and the resulting mechanical resp. electrical response of its pulvinated leaf system.

Part I is a review of previous investigations on the nature of the transmission process by which the excitation released during the process of stimulation is transmitted to the region where the resulting mechanical resp. electric response takes place, and of the theories which have been put forward to interpret the experimental results. Two types of interpretations have been proposed.

(*a*) The physico-physiological theory of Sir J. C. Bose, which was based upon a large number of investigations chiefly with two motile plants, *Mimosa pudica* and *Biophytum sensitivum*.

It was shown by him that the excitation is transmitted from the region of stimulation to that of response, as an electrical disturbance controlled by the same physiological laws as are found to govern the propagation of excitation in a nerve muscle unit. These include threshold and summation effects, repetitive responses to stimulation, electrotonus and polar effect of steady currents; effect of applications of cold, of stimulants and depressants. The responses observed both in plant and in animal tissues appeared to be satisfactorily explained on a physico-physiological theory of transmission of excitation, in the form of electric action potentials propagated by means of local currents.

(*b*) The chemical transmission theory is based upon the observations of Ricca, which were confirmed and extended by Snow, Haberlandt, Umrath and others. It is based upon the discovery and isolation from the expressed sap of *M. pudica* and other plants of substances which, when introduced to the cut-end of stem of a Mimosa leaf system, induce responses in it, viz. the fall of the pulvinus and closure of leaflets (Mimosa effect), which is identical with that produced by an ordinary physical stimulus. According to this theory stimulation results in the release of an irritability substance which is carried by means of a transpiration current to the motile pulvinus of the sensitive plant and which causes the characteristic response.

(*c*) It is shown how the two apparently contradictory interpretations can be reconciled by means of a physico-chemical theory of transmission of excitation along the plant tissue similar to one accepted at present for the explanation of the transmission of excitation in nerve muscle units. It is now known that in the latter case a chemical mediator, acetyl choline, plays an important rôle; in the case of the motile plant tissue the irritability substance plays a similar rôle.

Part II contains an account of the attempts which have been made by different investigators to isolate the irritability substance and determine its chemical structure. Using three different methods of extraction we have isolated two different substances, which give the Mimosa test. One contains an amino acid, a phenolic hydroxide and a free carboxalic group, while the other contains tannin associated with a glucoside. The limiting concentrations at which they give the irritability test is being determined.

Part III contains an account of our investigations on the nature of the so-called 'Active Substance' detected by Bose, by method of differential staining, in the pulvini of plants with motile organs. According to Bose a highly oxidizable substance is present, in concentrations proportional to the activity of the motor organ, i.e. to its amplitude and angular velocity of movement. The substance is neither a fat nor a lipoid body, but contains unsaturated carbon atoms in double or triple bonds.

Our investigations lead to the finding that a Crocin-like substance, viz. a glucoside of Crocetin, is present in these pulvini, similar to that found by Kuhn and Moewus in the motile flagella of the alga *Chlamydomonas eugamatos*. Since Crocetin is a carotinoid compound with conjugated double bonds, it is very probable that this Crocin-like substance is identical with the active substance found by Bose in the motile pulvini.

Part IV gives an account of our investigations on the action of the irritability substance extracted from Mimosa and other plants in producing the Mimosa effect. The transmission of excitation between two Mimosa plants, the cut-ends of whose two branches are connected by different kinds of water bridges, has also been investigated. It is found that only in rare cases a diffusion of the irritability substance takes place across the water bridge from the stimulated to the responsive plant.

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